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Spoilage of Fish in the Vessels at Sea
7. Further Studies on Seasonal Variations in the Landed Quality of Gutted, Trawler-Caught Atlantic Cod and Haddock[1,2]

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ABSTRACT

The same seasonal variations in the landed quality of trawler-caught Atlantic cod and haddock observed in 1956/57 have been found to occur during similar observations made in 1959.

The poorest quality, at the time of landing, were the fish caught in the summer and in November and early December. The better quality fish were landed during late winter, spring, and early fall.

These results were confirmed by independent observations made on trawler-caught fish at another Nova Scotian fishing port.

INTRODUCTION

The observations of experienced plant foremen and trawler fishermen indicate that the landed quality of iced cod and haddock are subject to considerable variation. They also suggest that, in part, these variations in landed quality are associated with the seasons of the year, or with some change in the living fish that occurs at different seasons of the year. Stated briefly, these men believe that they are able to land a much greater proportion of top quality fish during the winter and spring than in the summer and fall.

These observations of the fishermen were confirmed by measurements on the landed quality of trawler-caught cod and haddock during the period December 1956 to the end of December 1957 (Castell *et al.*, 1959). By comparing the quality of fish stowed in the vessels for similar periods, it was found that poorer quality fish were landed during the cold months of November and December and also during the warmer months of June, July and August. The best quality fish were landed during the months of February, March, April and September. This seasonal spoilage pattern was similar for cod and haddock. It was also observed in fish taken from eight individual trawlers as well as from the fleet as a whole.

Immediately the question arises: "Is this seasonal spoilage pattern a constant occurrence, or does it fluctuate or differ from year to year?" During the period March 1959 to January 1960 inclusive, another series of 3170 fish was examined and tested. The fish were taken from the same fleet of trawlers fishing out of Halifax. In this paper the results obtained are compared with the results of the previous series of tests.

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[2]Part 6 of this series appeared in this Journal, **16**(2): 223–233, 1959.

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EXPERIMENTAL METHODS

The experimental procedures were similar to those given in the previous paper in this series (Castell *et al.*, 1959). In brief, they were as follows: Large haddock and market cod were picked indiscriminately (except that no fish against the boards were used) from the trawler's pens at the time they were being discharged at the wharf. Ten similar fish were taken from each day's catch. They were filleted immediately, and the fillets were quick frozen in a contact freezer, packed in boxes, and held at −12°C until tested. All trimethylamine (TMA) determinations and the organoleptic grading of the fish were carried out on the defrosted fillets. The method of Dyer (1945, 1950) was used for measuring TMA, except that the extracts used were obtained by grinding whole fillets in a Waring Blendor with twice their weight of water.

In this particular series of tests, a very careful organoleptic examination was carried out on every fillet tested, with the object of assigning a grade based on the extent of deterioration that had taken place. Those fillets with no observable signs of deterioration (off-odours, discoloration, etc.) were graded I. Those that were judged to be merchantable, but not grade I, were classed as grade II. These included fillets with slight off-odours, slight discoloration or other indications of spoilage. Unmerchantable fillets with pronounced or disagreeable signs of deterioration were graded III. Among the 3170 fillets examined, 16 were graded III. As these were not very much worse than some of the poorer quality grade II fillets, it was decided, for statistical purposes, to include them in the grade II group. The result of the grading, therefore, was to divide the fish into grades I and II.

Previous experience had shown that most well-iced fish stowed in the trawlers for 1 to 5 days showed relatively little deterioration. For this reason the fish tested and examined were confined to those that had been iced in the trawlers for 6 to 9 days at the time of landing.

EXPERIMENTAL RESULTS

Trimethylamine (TMA) Values

Table I. Mean TMA values for 6- to 9-day-old gutted cod and haddock, tested immediately after discharge from Atlantic deep-sea trawlers. The first lot of 5110 fish was tested during the period November 1956 to November 1957 inclusive; the second group of 3130 fish was tested during the period March 1959 to January 1960.

Days in ice	No. of fish (1956/1957)	Mean TMA (1956/1957)	No. of fish (1959/1960)	Mean TMA (1959/1960)
6	2050	1.05	940	0.92
7	1510	1.35	1240	1.34
8	1170	1.90	730	1.77
9	380	2.56	220	2.40

Table I gives the mean TMA values of 6- to 9-day-old fish that were examined during the 1956/57 and 1959/60 test periods. It can be seen that in these two test periods the mean values for fish stowed for the same number of days in ice are quite similar. Figure 1 shows the mean monthly TMA values for the 7- and 8-day-old fish landed in 1956/57 and in 1959/60. The gap in one curve was the result of an insufficient number of tests to give significant results. It can be seen, however, that in general the curves are the same for the two test periods.

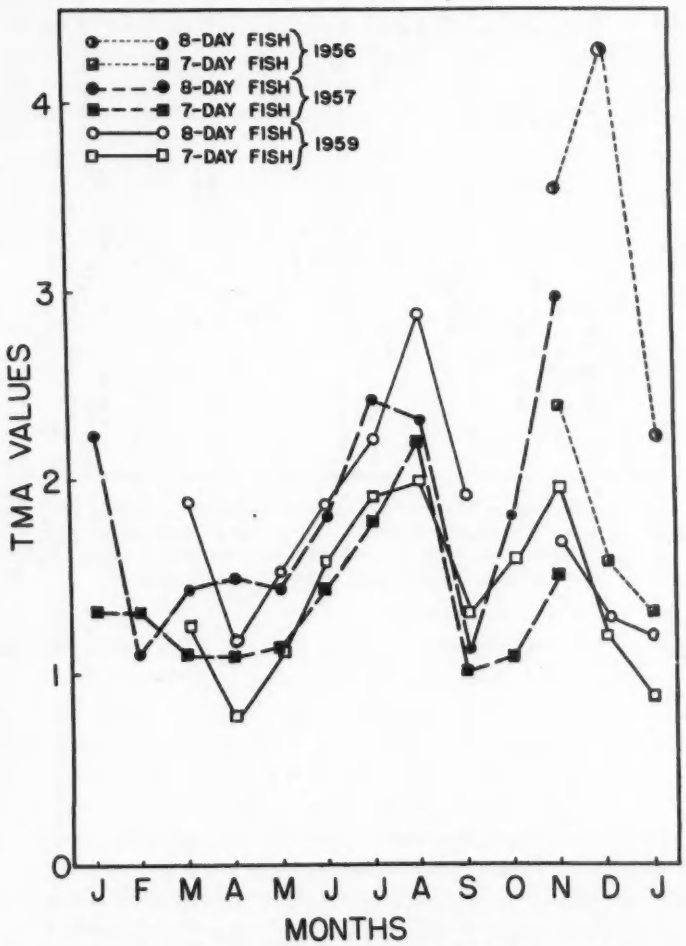

FIG. 1. Mean monthly TMA values of gutted cod and haddock that were iced for 7 and 8 days in the trawler's hold at the time of landing. It can be seen that there is a considerable similarity in the general slope of the curves for the different years.

The curve for the 7-day-old fish is probably the most representative, as it is based on a larger number of fish than those from the other storage-age categories, and they are a little more evenly distributed throughout the year. Further details showing the range and distribution of the TMA values for the 7-day-old fish

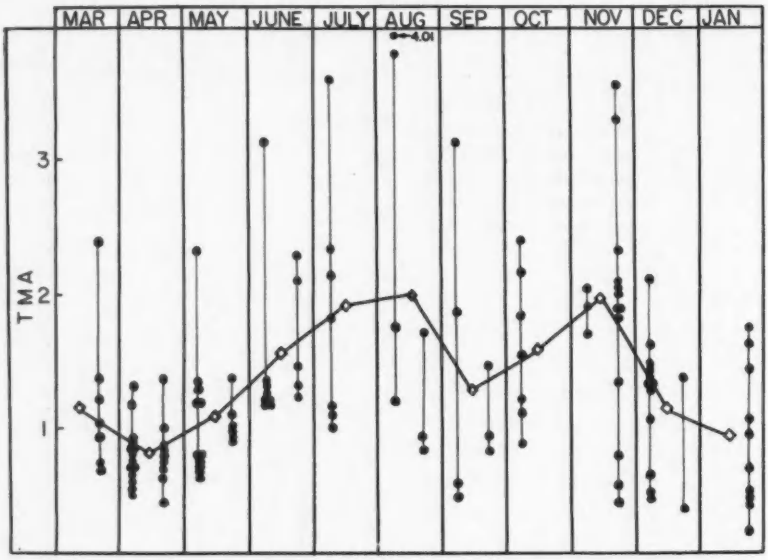

FIG. 2. Distribution of the TMA values for individual catches of 7-day-old fish, recorded at fortnightly intervals. The horizontal curve shows the mean monthly values for the same fish. During the spring months the individual values are grouped more closely around the means.

are given in Fig. 2, which shows the mean monthly TMA values and the scatter about the means for the individual catches taken at fortnightly intervals. Each individual point on the half-monthly vertical lines is a mean TMA value for one group of ten similar fish taken from one pen, representing a day's catch. In almost every month one or two trawlers had a "bad" trip, resulting in poorer quality fish with much higher than average TMA values. It can be seen that during March, April, May, and the first half of June, the values for the individual catches were more closely grouped around the monthly means. The higher average values during the summer and most of the fall were accompanied by a wider spread in the values for the individual catches.

This graphic picture showing the distribution of the TMA values at different seasons can be expressed more precisely: the mean value for all 7-day-old fish caught during March, April and May is 0.95, with a standard deviation of 0.41.

For the period of June, July and August both the mean and the standard deviation are approximately doubled, being 1.80 and 0.90 respectively.

Organoleptic Grading

Up to this point, the criterion used for indicating the degree of spoilage has been the accumulation of TMA in the muscle. In addition to this, an organoleptic grade was given to every fillet tested. Figure 3 compares the monthly percentages of grade I and grade II fish with the monthly mean TMA values. As might be expected, when the TMA values rose, the percentage of grade I fish decreased. The overall seasonal spoilage pattern obtained from measuring TMA was similar to that obtained by organoleptic grading of the fillets.

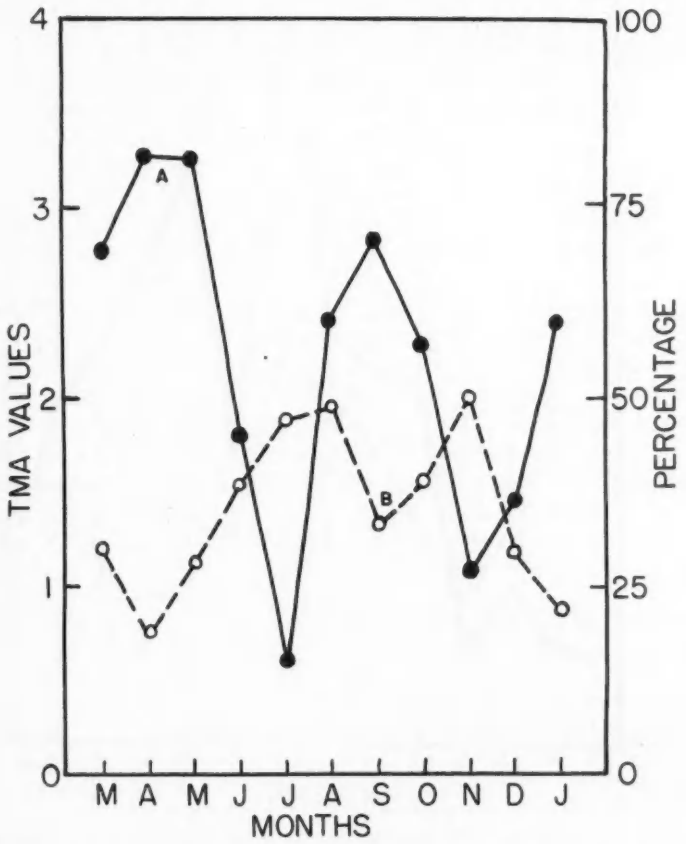

FIG. 3. Mean TMA values (B) and the percentage of grade I fish (A) for 7-day-old fish examined during the 1959/60 test period.

Results from Lunenburg. While this second series of tests was being carried out with fish landed at Halifax, the Lunenburg Sea Products Ltd., Lunenburg, N.S., was independently carrying out a somewhat similar series of 2400 tests with trawler-caught fish landed by other vessels at the fishing port of Lunenburg.

Their method of selecting the fish to be tested was not the same as that used in our tests. It tended to stress the poorer quality fish landed by their trawlers, rather than a random selection from specific storage-age groups. Nevertheless it can be seen from Fig. 4 that the seasonal changes in quality observed at Lunenburg coincide with those observed at Halifax.

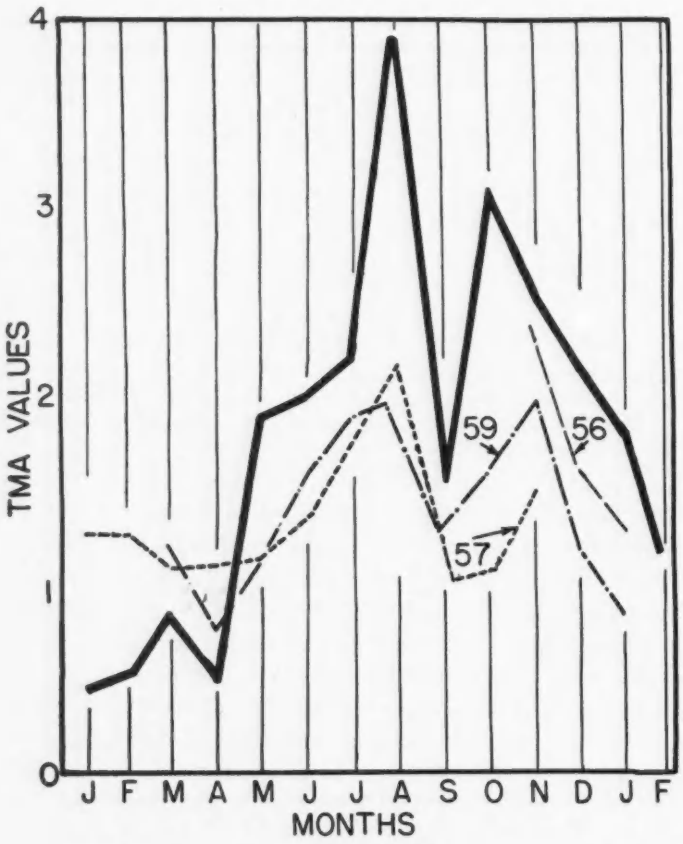

FIG. 4. Monthly mean TMA values (heavy line) for the 2400 fish tested at Lunenburg (see text for details) compared with similar TMA-value curves for the 7-day-old fish tested in Halifax during portions of 1956, 1957 and 1959.

It is also of interest to read the summation of the results of their observations on the seasonal changes in the quality of the cod and haddock landed in Lunenburg during 1959:

> "The year 1959 can be divided into three parts (in regard to the quality of the fish landings) namely: 1) January to April—a time of top quality fish when all boats are landing high percentages of cod and haddock; 2) May to August—a time of poorer quality fish, when some boats are landing large catches of flounder and redfish (and therefore less cod and haddock); 3) September to December—the quality during September is fairly well up. There is a sharp drop in October and a rise again in December."

CONCLUSIONS

Comparison of the landed quality of gutted cod and haddock in 1959 and part of 1960 has shown the same seasonal fluctuations that were previously observed in 1956/57. Better quality fish are landed during the late winter, spring, and early fall than during summer and late fall. A similar pattern was obtained by independent observations made in another fishing port during 1959. This strongly suggests that the pattern observed is a normal occurrence and can be expected to repeat itself under similar conditions.

Examination of the data from individual catches shows that during seasons of relatively poor quality landings, the increase in mean TMA values is accompanied by an increase in the standard deviation. This indicates that in spite of the drop in the quality of the fish as a whole, some vessels are also landing comparatively good fish.

Seasonal fluctuations in the quality of the landed fish, observed by using TMA values, were confirmed by careful organoleptic grading of the fillets.

From a commercial standpoint it is interesting to note the changes in the percentage of grade I fish landed at different periods of the year. During the late winter and spring months, approximately 75% of the fillets cut from the 7-day-old fish were free from off-odours and were graded I. During the warm summer months, and again in November, the amount of grade I 7-day-old fish dropped to 25% or less.

In addition to the fact that seasonal changes occur in the spoilage pattern of these fish, there is also the more fundamental problem of establishing the cause or causes of these changes. Speculation on these causes was given in the previous paper of this series (Castell *et al.*, 1959). No additional information has been obtained that would lead to any firmer conclusions than the suggestions given there.

Note. In an accompanying paper Castell *et al.* (1961) describe results of a further study on the relation between TMA values and the organoleptic grades given to these same fish. It was found that during the summer and late fall, fillets with a given TMA level had a lower organoleptic rating than fillets with the same TMA level caught in the spring or late fall. This does not alter the overall picture of the seasonal variations in the landed quality of the fish that have been recorded here. However, it does indicate that if the TMA values were to be transposed to corresponding organoleptic ratings, many of the fish caught in summer and late fall would be even poorer in quality than the observed rise in the TMA values would indicate.

ACKNOWLEDGMENT

This work could not have been done without the co-operation of the management and staff of the Sea-Seald Division of the National Sea Products Ltd. at Halifax. For this we are sincerely grateful.

We also wish to express our thanks to F. C. Read, chemist, and to the management, of Lunenburg Sea Products Ltd., Lunenburg, N.S., for granting us permission to include in this paper the results of the tests made at that plant.

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Grading Fish for Quality
4. Variations in the Relation Between Trimethylamine Values and Grades for Gutted, Trawler-Caught Atlantic Cod and Haddock[1,2]

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ABSTRACT

The accuracy of the trimethylamine (TMA) grade probability curve, developed in 1958 by Hoogland for gutted, trawler-caught Atlantic cod and haddock has been verified by tests made on 3170 cod and haddock.

It has also been shown that the relation between TMA and grade is not the same for cod as it is for haddock. For a given TMA level, haddock show greater deterioration.

The relation between grade and TMA also changes with the season. For a given level of TMA, fish caught in the summer and late fall usually show more deterioration than those caught during the spring months.

INTRODUCTION

During previous studies on the grading of fish, Hoogland (1958) developed a probability curve showing the relation between organoleptic grade and trimethylamine (TMA) values for gutted, trawler-caught Atlantic cod and haddock. This was based on data obtained from grading of several hundred fish carried out at this Station by a group of experienced fish-plant foremen. Since that time we have had occasion to grade and to make TMA determinations on fillets cut from an additional 3170 fish.

The data, obtained from grading and testing this large number of fillets cut from fish taken directly out of the trawlers, have provided an opportunity of verifying the curve developed by Hoogland. Because the species and the date of landing for each fish had been recorded, it was possible to develop separate curves showing the TMA–grade relations of cod and haddock separately, as well as for fish caught at different seasons of the year.

EXPERIMENTAL PROCEDURES

Large haddock (3 to 7 lb) and market cod (3 to 8 lb) were all taken from the trawlers at the time of unloading at the wharf. Within a very short time the fillets were removed by experienced cutters, frozen in a contact-type quick freezer, and delivered to this Station by truck, where they were held in frozen storage until tested. This procedure was followed to prevent deterioration of of the fillets between the time the fish were removed from the trawler and the fillets were tested.

Storage in the freezer was limited to the shortest time compatible with the time schedule for testing. Usually this was from 1 to 48 hours. At certain

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periods, however, when large numbers of fish were being recovered, the storage of some fillets was extended up to but never beyond 14 days. Control tests showed that fillets stored under these conditions showed no significant change in weight or TMA values at 14 days.

Before testing, the fillets were thawed by leaving them at room temperature in waxed cardboard boxes. During this stage, and particularly when the last traces of frost were leaving, the fillets were repeatedly examined and smelled by at least two, and more often three persons in the laboratory who had had considerable experience in grading fillets. Because of the conditions under which the fish had been selected there were very few spoiled, i.e. grade III, fish. The problem of grading was chiefly one of determining whether the fillets showed no observable deterioration (grade I) or were in the early stages of spoilage where the first off-odours were beginning to develop grade II.

The TMA determinations were always made by grinding the whole fillets with twice their weight of water in a gallon-size Waring Blendor and then testing by the colorimetric procedures given by Dyer (1945, 1950).

Table I condenses the TMA values and the grading results of all the fish tested. It is given both to present a general picture of the results obtained and also to show the procedure used to develop the curves relating TMA values to

TABLE I. Percentage of fillets in grades I and II in various TMA ranges between 1.0 and 10.0, for both cod and haddock, taken over the 11-month test period.

TMA ranges	Number of fillets			Percentages	
	Grade I	Grade II	Total	Grade I	Grade II
0.00–0.40	409	92	501 (263)*	82	18
0.41–0.60	302	80	382 (207)	79	21
0.61–0.80	258	98	356 (171)	72	28
0.81–1.00	241	136	377 (197)	64	36
1.01–1.20	152	136	288 (125)	53	47
1.21–1.40	104	129	233 (90)	45	55
1.41–1.60	92	117	209 (94)	44	56
1.61–1.80	53	107	160 (71)	33	67
1.81–2.00	46	102	148 (95)	31	69
2.01–2.20	27	68	95 (52)	28	72
2.21–2.40	20	63	83 (39)	24	76
2.41–2.60	15	65	80 (37)	19	81
2.61–2.80	8	42	50 (27)	16	84
2.81–3.00	5	38	43 (27)	12	88
3.01–3.20	6	21	27 (12)	22	78
3.21–3.40	2	18	20 (9)	10	90
3.41–3.60	3	13	16 (12)	19	81
3.61–3.80	1	18	19 (10)	5	95
3.81–4.00	2	12	14 (7)	14	86
4.01–5.00	2	34	36 (22)	5	95
5.01–6.00	3	20	23 (9)	13	87
6.01–7.00	0	6	6 (1)	0	100
7.01–8.00	0	3	3 (2)	0	100
8.01–9.00	0	0	0 (0)	0	...
9.01–10.00	0	1	1 (0)	0	100

*Figures in parentheses refer to numbers of haddock only.

the percentage of fish in each of the grades. This same procedure has been used for treating the results obtained from the cod and haddock separately as well as for fish taken at the different seasons of the year. In addition to the total numbers of cod and haddock in each of the TMA ranges (column 4 of the Table) an additional set of figures has been added in parentheses. This is the number of haddock only. From this it can be seen that in each of the TMA ranges there were approximately equal numbers of cod and haddock.

Figure 1 shows the TMA-grade probability curve developed by Hoogland (1958) for mixed, gutted, trawler-caught cod and haddock taken throughout the fishing season. Superimposed upon this curve are the results obtained

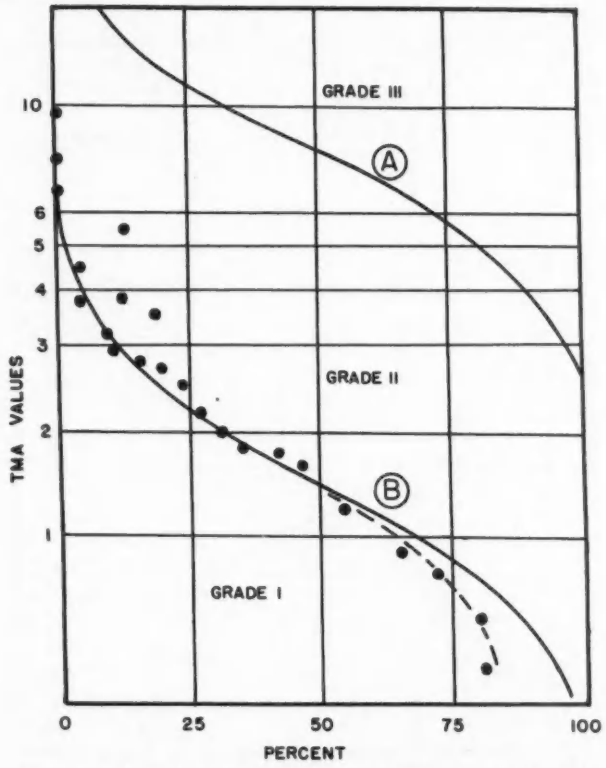

FIG. 1. "A" and "B" are the probability curves developed by Hoogland, showing the relation between TMA values and grades for gutted, trawler-caught Atlantic cod and haddock. The black circles show TMA values and percentage distribution in grades I and II for 3170 6- to 9-day-old cod and haddock. The number of observations represented by each of these points is shown in column 4 of Table I.

from 3170 mixed cod and haddock that were tested and graded in the period March 1959 to the end of January 1960. It can be seen that they follow very closely along the curve developed by Hoogland.

The curve in Fig. 1 is based on the combined results of 1581 tests made on fillets from haddock and 1589 tests made on fillets from cod. These, in turn, can be subdivided into:

Haddock:	grade I — 750;	grade II — 831
Cod:	grade I — 1001;	grade II — 588.

This shows a fairly even distribution, both in regard to species and to grades in the fish that were examined. Figure 2 separates the results obtained from the cod and the haddock, and includes Hoogland's curve as a reference. It

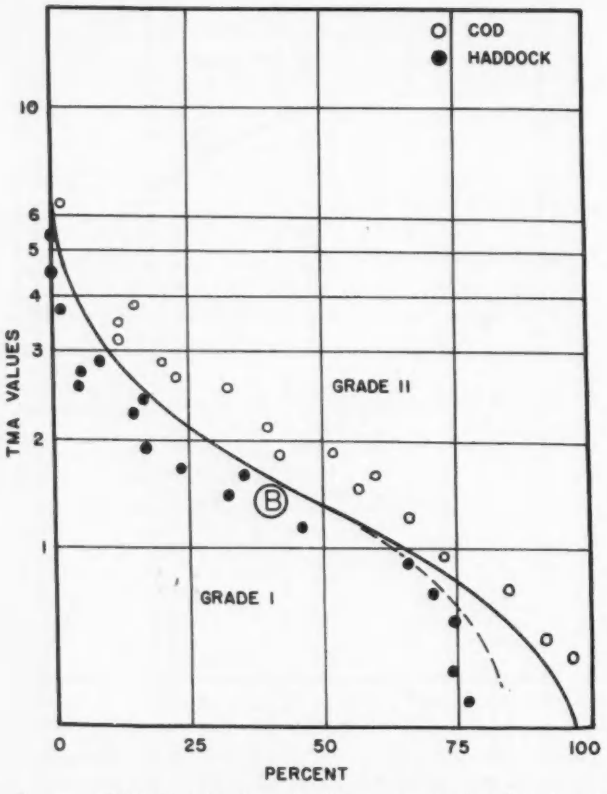

FIG. 2. "B" is Hoogland's probability curve showing the relation between TMA values and grades for mixed cod and haddock. The data points surrounding this curve were obtained from the same fish that were used in Fig. 1, except that the cod and haddock have been treated separately.

is very obvious that haddock were down-graded at lower TMA levels than cod. For example, at a TMA level of 1.0, only 55% of the haddock were grade I, while at the same level close to 70% of the cod were grade I.

It has been shown previously that the spoilage pattern of these trawler-caught Atlantic cod and haddock was not the same throughout the year. At the time of landing, the winter, spring and early fall fish were in a better state of preservation than the corresponding fish caught in the summer and late fall months (Castell *et al.*, 1959; Castell and Giles, 1961), In addition to affecting the rate of spoilage, there probably were also slight differences in the course of spoilage at different periods of the fishing season. This leads one to question whether a given TMA value has the same significance, in terms of the extent of spoilage that has occurred, at different seasons of the year.

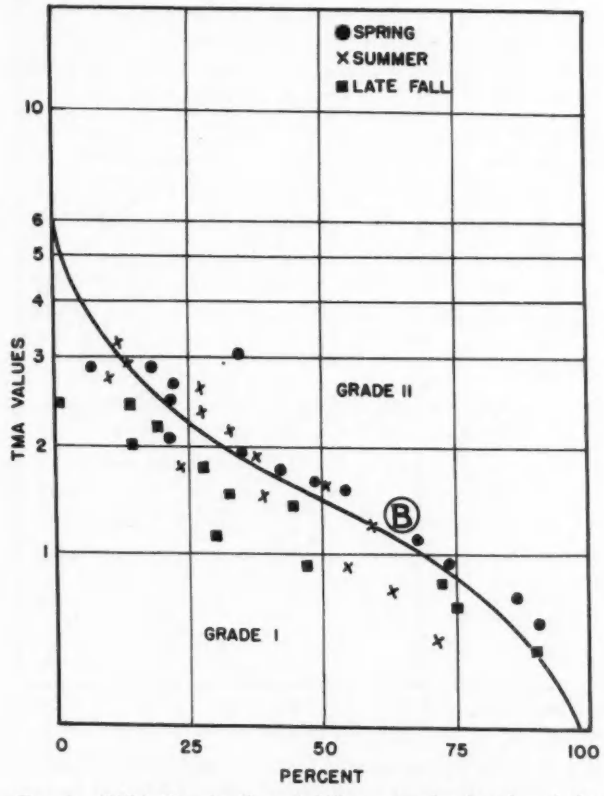

FIG. 3. "B" is Hoogland's probability curve showing the relation between TMA values and grades for mixed cod and haddock. The data points surrounding this curve were obtained from the same fish as those used in Fig. 1, except that the fish caught in the spring, in the summer, and in the late fall have been treated separately.

Again using Hoogland's curve as a background, the TMA–grade relations for mixed cod and haddock have been compared for fish landed in the spring, summer and late fall.

It can be seen from Fig. 3 that fish caught at different seasons of the year did not have the same TMA–grade relation. During the spring, when larger catches and better quality fish were being landed, a given TMA level indicated a higher quality fish than the same level for the similar fish caught in the summer or late fall.

It is interesting to observe what happens when the effects of species and season on the TMA–grade relation are combined. This is illustrated in Fig. 4

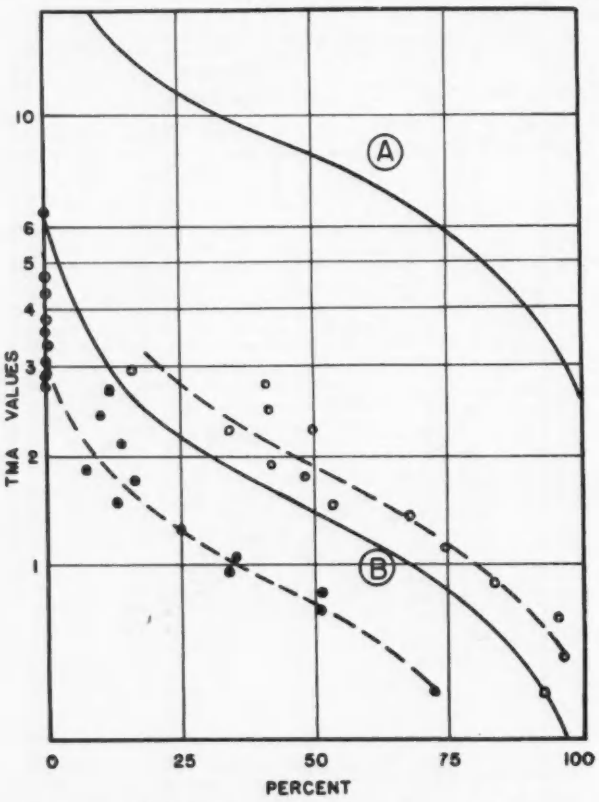

FIG. 4. TMA values and the percentage distribution in grades I and II for cod caught in the spring (open circles) and haddock caught in the summer (closed circles). These fish were all trawler caught and were in ice from 6 to 9 days at the time of landing. Hoogland's curve "B" is added to the Figure for reference and comparison.

which gives the data for cod, caught in the spring, with haddock, caught in the summer. Under these conditions it can be seen that haddock with a TMA value of 1.0 would have about 35 chances out of 100 of being grade I while a cod with the same TMA level would have 80 chances in 100 of being grade I.

DISCUSSION OF RESULTS

This seasonal shifting relation between grade and TMA value apparently complicates the problem of obtaining an objective measurement for the quality of fish. But in point of fact, after examining these results, the fishery officers, who had been continuously grading fish on the wharf, have been emphatic in their reaction: "This certainly clarifies the problem for us and demonstrates what we already know to be so!"

It also underlines the suggestion that "Although objective measurements can often be very useful as references, they should not be used as the bases for defining commercial grades of fish."

It might be objected that all the results given in this paper deal only with strictly fresh fish or those in the very early stages of spoilage. No data are presented to verify the other line in Hoogland's TMA–grade probability curves—that is, the line separating grades II and III. This is a justifiable criticism and it would have been of real interest to see what happened to these same relationships during the later stages of spoilage.

This occurred simply because too few grade III fish were encountered among those that were tested to give results that would have meaning.

One other point is worth mentioning. The TMA–grade relations that were found for the mixed cod and haddock during the whole test period, agree very well with the probability curve developed by Hoogland (Fig. 1). There is one small portion, however, where they do not agree. This is in the region of the very low TMA values at the lower, right-hand end of the curves. Hoogland's curve indicates that there should have been greater percentages of grade I fish than were actually found. If the fish that were tested had included some that were 1 to 3 days in ice, this difference would not have occurred, since a higher percentage of very fresh fish with low TMA values will be graded I than would be found with 6- to 9-day-old fish with similar low TMA values.

It would seem very probable that for unselected fish, taken indiscriminately from the trawler's catch, this region of Hoogland's curve gives the truer picture. It also indicates the care that is needed in applying a probability curve to a specifically selected group of fish such as these older cod and haddock with very low TMA values.

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Surface Temperature and Salinity off the Washington and British Columbia Coasts, August, 1958 and 1959[1]

By Felix Favorite

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ABSTRACT

Oceanographic data collected off the Washington and British Columbia coasts during August, 1958 and 1959, through Canadian and United States research programs provide the best synoptic coverage attained in this area. Comparison of August surface data for the two years shows that the effects of local runoff can be traced over several hundred miles offshore. This implies, but does not necessarily confirm, the absence of any appreciable net surface current parallel to the coast northward of the Strait of Juan de Fuca to Dixon Entrance, in summer.

The 1958 observations were made at the time of the anomalous Fraser River sockeye run through Johnstone Strait and provide a good picture of the existing coastal and offshore oceanographic conditions. It is suggested that the seaward extent of dilute surface water may determine the location where homeward migrating salmon enter coastal waters.

INTRODUCTION

Sockeye salmon (*Oncorhynchus nerka*) returning to spawn in the Fraser River, British Columbia, have a choice of two routes around Vancouver Island, from the north through Johnstone Strait or from the south through the Strait of Juan de Fuca. The latter is considered the normal route, but in 1958 practically the entire run was reported to have approached Vancouver Island from the north, and an unprecedented percentage of an unusually large run proceeded through Johnstone Strait (Internat. Pacific Salmon Fish. Comm., 1959).

This behaviour may have been due to a progressive northward intrusion of warm water off the Canadian coast from 1955 to 1958 as shown on the sigma-*t* surface 26.60 by Tully *et al.* (1960).

Alternatively, it could be due to an unusual change in position or extent of the local runoff flowing out of Queen Charlotte Sound and the Strait of Juan de Fuca. The migration pattern of the Fraser River salmon run returned to normal in 1959 (Internat. Pacific Salmon Fish. Comm., 1960). The purpose of this report is to present the surface conditions off the Washington and British Columbia coasts during August, 1958 and 1959, and to determine if any significant differences occurred in these two years that could have affected the 1958 migration route of the salmon.

Barnes and Paquette (1954) have described the general circulation off the Washington coast and noted that the brackish water originating in the outflow

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of the Columbia River could be detected over 200 miles offshore. Doe (1955), presenting data from project "Offshore", has described the origin and identification of water masses off the British Columbia coast, and detected brackish water several hundred miles offshore which he believed originated in local runoff.

SURFACE DATA, 1958

At the time the Fraser River salmon run was entering coastal waters in 1958, there were numerous oceanographic cruises being conducted in the area. The M.V. *Attu* and M.V. *Pioneer*, chartered by the Seattle Biological Laboratory of the U.S. Bureau of Commercial Fisheries to conduct experimental fishing and oceanographic research for the International North Pacific Fisheries Commission, were returning to Seattle, having completed operations in the Aleutian area (Favorite and Pedersen, 1959). At the same time, the Pacific Oceanographic Group of the Fisheries Research Board of Canada was making observations from C.N.A.V. *Whitethroat* and C.N.A.V. *Oshawa* (Fisheries Research Board of Canada, 1958 a, b). Also, the Oceanographic Laboratories of the University of Washington, Seattle, Washington, were independently conducting an oceanographic cruise in R.V. *Brown Bear* (Fleming *et al.*, 1959). Figure 1 shows the locations of all surface observations made from these vessels in the area under consideration during August 1958. The observed surface salinity and temperature distributions are shown in Fig. 2.

Temperature

The temperature distribution (Fig. 2) shows three interesting features: The region of divergence of the isotherms in the vicinity of Lat. 50°N, Long. 135°W; the band of cool water along the coast; and the apparent northward intrusion of warm water seaward of the cold water along the coast.

The divergence of the isotherms appears to be associated with a tongue of dilute water extending seaward, and is not to be confused with the divergence of the Westwind Drift which occurs farther offshore. The band of cold temperatures along the coast has been explained by Tully (1937) and Barnes and Paquette (1954) as a normal late summer condition, and has been attributed to upwelling.

Reference is made only to the August, 1950 and 1951, data presented by Doe (1955) and the average August conditions presented by Robinson (1957) in determining whether or not the 1958 conditions were anomalous. Although limited data are available for other years, only the above data are considered to be adequate enough to permit comparison. The 1951 temperatures are representative of average conditions and the 1958 temperatures were approximately 1 to 2 C° higher than average; however, the general configuration of the isotherms was similar.

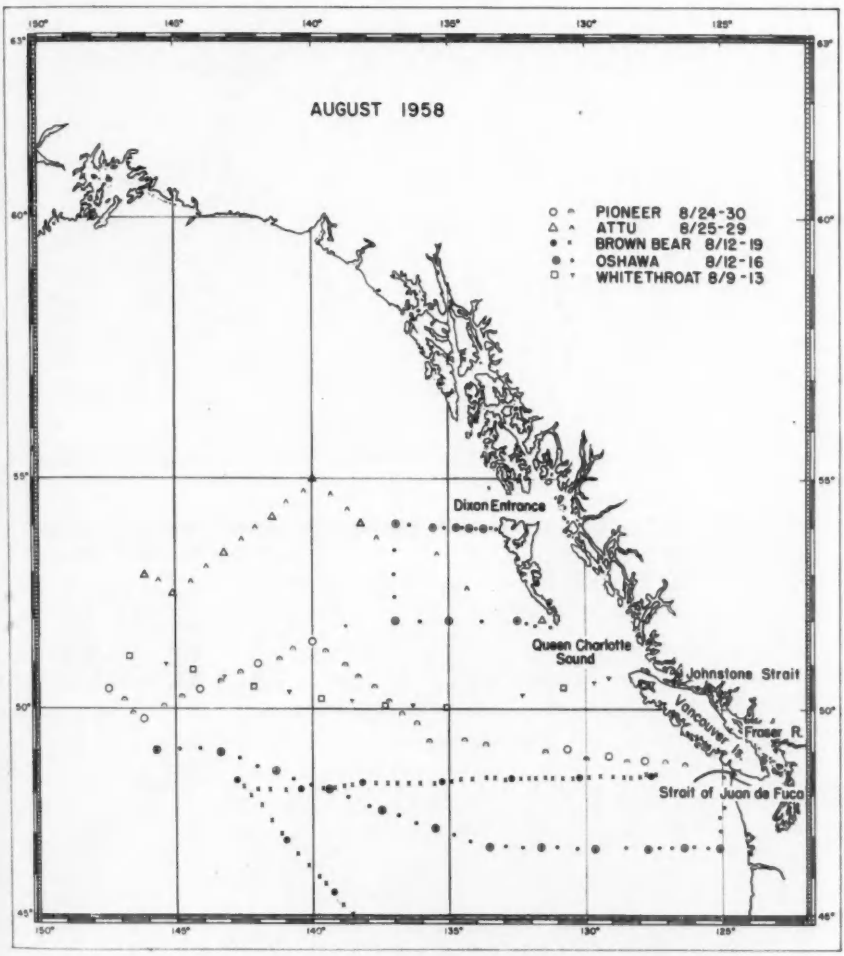

FIG. 1. Oceanographic and bathythermograph stations, August 1958. (Large symbols indicate oceanographic stations).

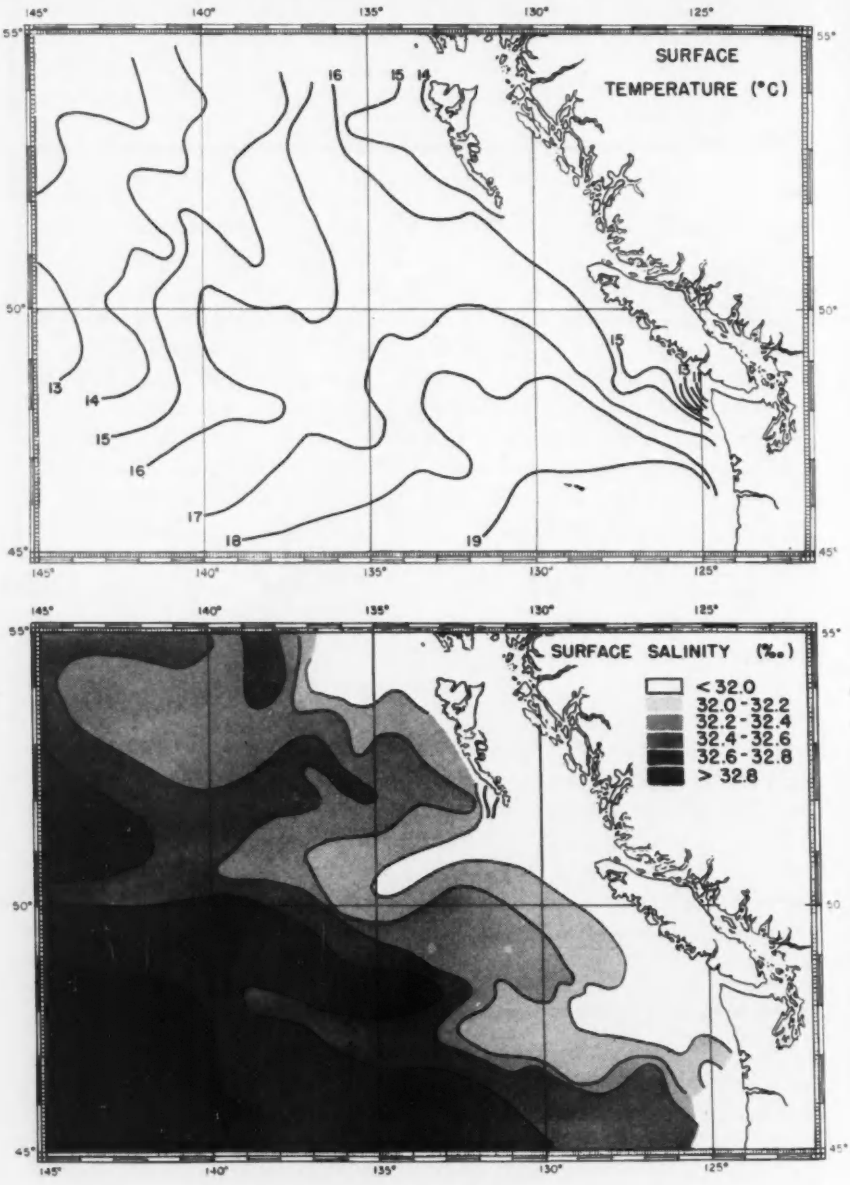

FIG. 2. Surface temperature and salinity, August 1958.

Salinity

The effects of local runoff from the Strait of Juan de Fuca, Queen Charlotte Sound, and Dixon Entrance are immediately apparent as excursions of dilute water into the coastal and offshore regions. These occur as three distinct tongues normal to the coast, and can be identified several hundred miles offshore. The protrusion of the 32.0‰ isohaline off Queen Charlotte Sound extended to Lat. 50°N and Long. 135°W, coinciding with the divergence of the isotherms, as noted above.

SURFACE DATA, 1959

The 1958 data showed that closely spaced observations are necessary to show continuity of the properties in the coastal region. Having been notified of the extensive 1959 summer cruise plans of the Pacific Oceanographic Group (Fisheries Research Board of Canada, 1959), personnel aboard the charter vessels *Pioneer* and *Tordenskjold* made additional observations along Lat. 51 and 53°N in August 1959 while en route to Seattle from the Aleutian area (Favorite *et al.*, 1961). Bad weather prevented taking as many observations as were planned. Figure 3 shows the locations of the surface observations, and the temperature and salinity distributions are shown in Fig. 4.

Temperature

The surface temperatures in 1959 were generally 2 to 3 C° less than in the previous year, and approximately 1 C° less than the average conditions shown by Robinson (1957). The offshore divergence of the isotherms occurred a little southward and nearer to the coast than in 1958. Even though a marked difference existed in the surface temperatures, the general configuration of the isotherms was similar to that of August 1958. The band of colder temperatures was again present along the coast, and the apparent northward intrusion of warm water was evident approximately 200 miles west of the Washington coast, but did not intrude northward past Vancouver Island as it did during the previous year.

Salinity

As in August 1958, the local dilution from Queen Charlotte Sound and Dixon Entrance was clearly evident off the coast, but the region of dilute water was confined nearer the coast along a front extending from Lat. 49 to 55°N about 300 miles offshore and approximately parallel to the coastline. Doe (1955) denoted this general area as the separation of the coastal and offshore water masses.

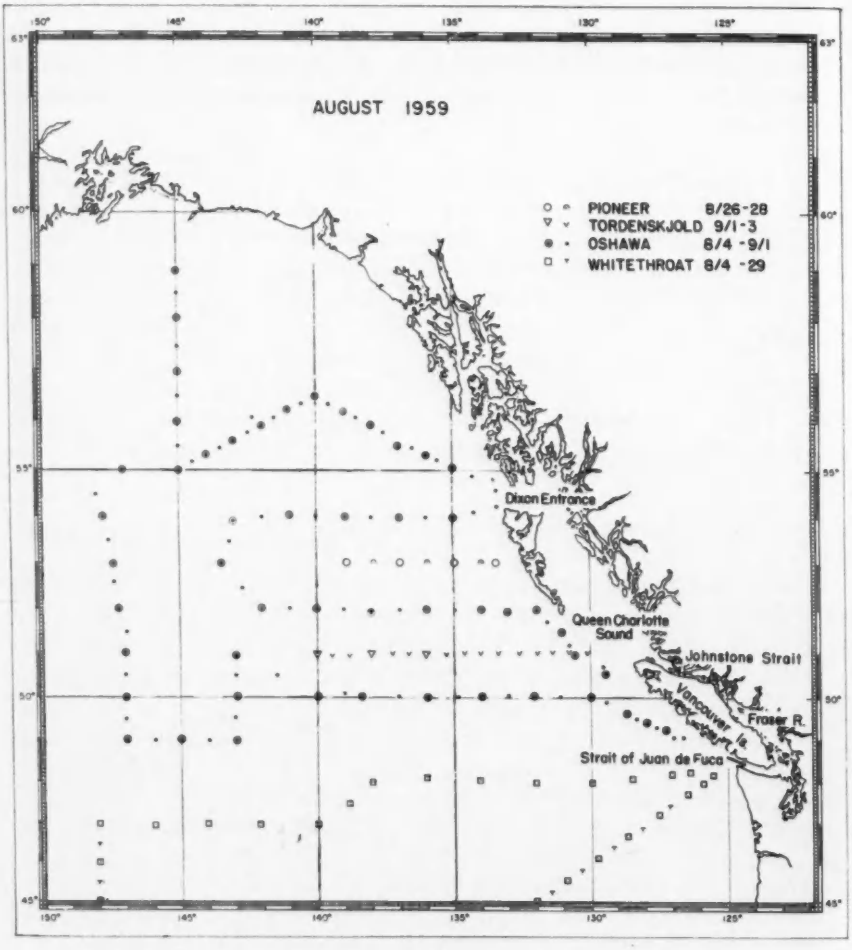

FIG. 3. Oceanographic and bathythermograph stations, August 1959.
(Large symbols indicate oceanographic stations.)

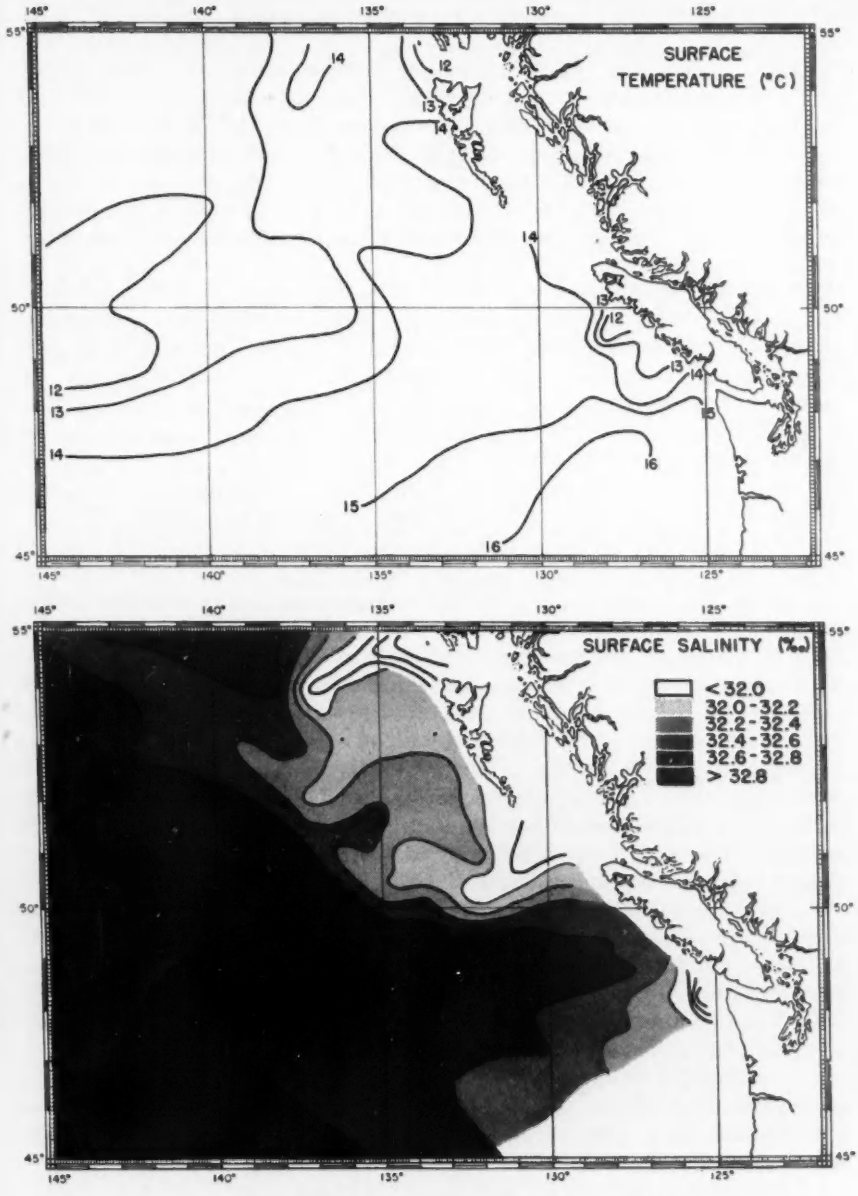

FIG. 4. Surface temperature and salinity, August 1959.

DISCUSSION AND SUMMARY

The coasts of Washington and British Columbia are mountainous, and snow-melt and precipitation draining in spring from an extensive watershed area result in the discharge of considerable amounts of fresh water into the coastal regions. After mixing with sea water in the inshore area, the runoff enters the ocean directly or through various straits or passes. An estimate of how far offshore the properties associated with the runoff may be present, can be ascertained by tracing the seaward extent of the coastal dilution as shown by the surface salinity.

The local runoff from Queen Charlotte Sound in August 1958, as shown in Fig. 2, can be traced across the coastal region into the offshore region, and existed several hundred miles further offshore than shown by previous data cited. Surface temperatures were approximately 1 to 2 C° greater than average. In 1959, although the runoff again was clearly evident offshore, it was confined nearer to the coast than in 1958. Surface temperatures were 2 to 3 C° less than in 1958, and approximately 1 C° less than average.

In both years it is apparent that the surface temperature is not a good indicator of the extent of location of the local runoff during summer. This may possibly be explained by the fact that the cold, snow-melt runoff enters the ocean in late spring; thus subsequent insolation during the summer would result in an equilibrium ocean temperature which prevents any further thermal identity of the water. This gradual warming would tend also to stabilize the layer of dilute water and help to maintain the identity of its salinity values.

In order to ascertain whether the temperature or the salinity more clearly reflected the surface circulation, the dynamic topography of the area was investigated. However, the limited number of stations providing data suitable for permitting this calculation, in contrast to the number of surface observations available, prevents a comparative picture. A similar lack of correlation between the surface temperature and salinity distribution appeared in Doe's work. He compared also the dynamic topography to isentropic charts and discovered that considerable difference in detail existed.

Then too, the excursion of the local runoff, as clearly defined tongues normal to the coast, is interesting, because it suggests the absence of any appreciable northerly or southerly current along the coast north of the Strait of Juan de Fuca to Dixon Entrance during the summer months. This is not confirmed by calculations of water transport, and the discrepancy can be resolved only by future direct current measurements.

In attempting to relate surface conditions in the ocean to the migration of salmon, one must be very cautious, because the factors which guide anadromous species to their parent streams after a 1- to 3-year residence in the ocean are not known (Talbot and Sykes, 1958).

The similarity between the location and seaward extent of the local dilution from the British Columbia and Washington coasts in August, 1958 and 1959, and the lack of knowledge concerning the response of salmon to variations in the

marine environment, prevent one from making any specific conclusions in regard to the effect of oceanographic conditions on the anomalous migration path of the 1958 Fraser River salmon run. Nevertheless, the extrusions of dilute surface water off the British Columbia coast provide an indication of the location in the ocean where the shoreward-migrating salmon first detect this dilution and may determine the point at which they enter the coastal waters.

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Observations on *Post mortem* Biochemical Changes in Fish Muscle in Relation to *Rigor mortis*[1]

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ABSTRACT

The influence of the degree of exhaustion of fish at death and their subsequent storage temperature on the relationship between *rigor mortis*, lactic acid formation and changes in certain acid-soluble phosphorus-containing fractions in muscle have been examined. The fish used were rainbow trout (*Salmo gairdneri*) and starry flounders (*Platichthys stellatus*) killed under controlled conditions in an aquarium and sockeye salmon (*Oncorhynchus nerka*) caught by gill net or seine net under commercial conditions. The majority of the fish contained little or no molybdate-labile P (phosphocreatine) at death, but in those that did (chiefly unexercised fish) the P of this fraction, sometimes after a short period of increase *post mortem*, decreased to a very low value. Acid-labile P (adenosine-triphosphate) decreased later *post mortem* than did phosphocreatine, and rigor developed during this period of decrease. Onset of rigor was detected at higher concentrations of acid-labile P at 20 than at 0°C. Acid-stable P decreased *post mortem*, but without showing the rather sudden disappearance from the muscle exhibited by the two labile fractions, the decrease continuing throughout rigor.

In unexercised fish the relationship between the course of rigor and lactic acid formation was distinctly different at 0 and 20°C. At 20°C the lactic acid concentration reached a maximum in the muscle by the time rigor was fully established or very soon thereafter. At 0°C accumulation was much slower and had only reached about 50% of its maximum concentration when rigor was fully established. Evidence was presented that the duration of full rigor is related to the continued production of lactic acid in the muscle, rather than to the quantity of lactic acid accumulated.

INTRODUCTION

Excellent descriptions of *rigor mortis* in fish, including observations of the effects of several variables on the time course of rigor, were published years ago (Ewart, 1887; Anderson, 1909). Since then numerous investigations have been made of the biochemical changes that occur in fish muscle within a few hours or days *post mortem*, but in only a few of these investigations have attempts been made to relate the changes to the course of *rigor mortis*.

Leim *et al.* (1927), Macleod and Simpson (1927), Ritchie (1927), Macpherson (1932), Sharp (1934), Locke (1935), Amano *et al.*, (1953), and Noguchi and Yamamoto (1955a, b) have studied the changes in glycogen and lactic acid content of fish muscle *post mortem*. Macleod and Simpson (1927) and Ritchie (1927) made observations of the relation between the changes and the course of

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rigor mortis. Ritchie found that while there was some increase in lactic acid concentration during rigor, there was little or none once rigor had disappeared and the findings of Macleod and Simpson tended to confirm this. Locke (1935) found that fish caught in the spawning season either failed to go into *rigor mortis* at all, or stayed in rigor for only a very short time. The quantity of lactic acid accumulated in spawning fish *post mortem* was abnormally low and did not at any time reach values found in non-spawning fish in good condition. Although Macleod and Simpson concluded that rigor can never become marked so long as glycogen remains in the muscle, the later findings of Macpherson (1932), Sharp (1934), Tarr (1949), and Noguchi and Yamamoto (1955a, b) indicate that this is not true, but rather that some might still be present during rigor, depending on the quantity of glycogen present at death and on the subsequent storage temperature.

Sharp (1931), Fujimaki and Kojo (1953), Heen (1954) and Saito and Arai (1957a) examined the changes that occurred in the concentration of phosphocreatine (molybdate-labile phosphorus) in fish muscle *post mortem*, and found that it decreased rapidly from the time of death. Fujimaki and Kojo (1953) reported that phosphocreatine disappeared from the muscle before full rigor was established. They also examined the changes that occurred in the acid-soluble phosphorus compounds of fish muscle during the first 8 hours *post mortem*, and found that the major change was a decrease in nucleotide phosphorus and in glucose-6-phosphate. Golovkin and Pershina (1957) investigated the changes that occurred in the acid-labile phosphorus of fish muscle at intervals of 1 or 2 days for a period of 10 days *post mortem*. Noguchi and Yamamoto (1955a) found that acid-labile phosphorus disappeared from fish muscle before *rigor mortis* was fully established. Jones and Murray (1957), Murray and Jones (1958), and Saito *et al.* (1957b, 1959a, b) made a detailed examination of the *post-mortem* changes in the nucleotides of fish muscle but the relation between these changes and the course of *rigor mortis* was not recorded.

In the present work we have investigated the influence of the degree of exhaustion of fish at the time of death and of the subsequent storage temperature on the course of *rigor mortis*, and also the relation between the course of *rigor mortis* as affected by these variables, the formation of lactic acid in the muscle, and the degradation of certain acid-soluble, phosphorus-containing fractions of the muscle. A previous investigation had indicated that the major early *post mortem* changes in phosphorus-containing compounds of sterile fish muscle was confined to the group of compounds that are soluble in acid (Tomlinson *et al.*, 1960). In addition, this group is known to include compounds (e.g. nucleotides, phosphorylated sugars, phosphocreatine) (Tarr, 1949) that are intimately related in warm-blooded animals and probably also in fish (Tarr, 1958; Gumbmann *et al.*, 1958; also *vide supra*) to muscular function and to *rigor mortis*.

METHODS AND MATERIALS

Fish

Kamloops trout (*Salmo gairdneri*) were obtained from the Cultus Lake, B.C., hatchery of the British Columbia Provincial Fish and Game Department. Starry flounders (*Platichthys stellatus*) were captured by beach seine in the Coal Harbour area of Burrard Inlet, B.C. The trout and flounders were held live in the Vancouver Public Aquarium until required. Sockeye salmon (*Oncorhynchus nerka*) were caught by gill net in the mouth of the Fraser River, B.C., and by purse seine at Point Roberts, Washington, U.S.A., under commercial fishing conditions.

Treatment of Fish Prior to Killing and Method of *Post-mortem* Storage

Trout and flounders were treated in one of three ways prior to killing. One group was captured (hand net) and killed by a blow on the head as quickly as possible. These fish are termed "unexercised" (Black, 1955). A second group was forced to swim vigorously for 30 minutes before being killed. These are termed "exercised". A third group was given the same treatment as the second group, followed by a period of struggling in air (10 minutes for trout, 20 minutes for flounders) before being killed. These are termed "exhausted". It was thought that the condition of the exhausted fish would probably correspond to that of most commercially caught fish on the Pacific coast. The flounders were difficult to exercise. They did not maintain a steady swimming rate as did the trout and for periods of time refused to swim at all. When left in air they struggled only spasmodically when disturbed.

Sockeye salmon were caught under commercial conditions, but records were kept of the condition of the fish on landing (e.g. lively, exhausted, dead) and of their subsequent treatment. Some were killed immediately after landing by a blow on the head, while others were allowed to die in air under the usual commercial conditions.

The circumstances under which all the fish died are recorded in the Tables.

Post mortem, pairs of fish as nearly alike as possible as to size and treatment immediately before killing were stored in the round, one of each pair in ice, the other at the prevailing temperature of the room or boat. These temperatures were recorded. The method of storage is indicated in the Tables. These two methods of storage are commonly used on this coast for fish that are later canned or frozen.

Sampling

All samples from trout and sockeye salmon were taken from the lateral-dorsal muscle, while those from flounders were taken from the muscle of the under side of the fish. Samples were frozen in liquid nitrogen immediately after being removed, placed in polyethylene bags, and stored at −30°C until they could be analysed. Each sample weighed 5 g or more. Samples were taken immediately

after the fish died and again after suitable intervals of storage. These intervals were so chosen that, as nearly as possible, samples representative of the onset of rigor, full rigor, softening, and complete resolution of rigor, were obtained.

Observation of *Rigor mortis*

Onset and duration of *rigor mortis* were observed by holding the fish by the head and noting the rigidity of the body (Cutting, 1939) and also by feeling the firmness and elasticity of the flesh.

Chemical Methods

Frozen samples (5 g) were weighed and, while still frozen, were homogenized in a Servall Omni-Mixer with 30 ml of 11.4% (w/v) ice-cold trichloroacetic acid (TCA). The homogenate was centrifuged at 18,000 g for 15 minutes at 0°C. The supernatant solution was decanted and stored at 0°C, then the residue was re-extracted twice with 30 ml of 10% (w/v) ice-cold TCA. These two extracts were combined with the first, and the residue was discarded.

Phosphorus fractions were determined, in duplicate, on the extracts as described below. All measurements of phosphorus were made by the method of King (1932):

(a) *Inorganic phosphorus* (I-P)

This was precipitated from an aliquot of the freshly prepared extract by the method of Mathison (1909); the precipitate was washed with ice-cold 2% ammonia solution and then dissolved in 5% TCA solution for estimation.

(b) *Molybdate-labile phosphorus* (ML-P)

This fraction, together with I-P, was determined on a second aliquot of the freshly prepared extract by the method previously described (Tomlinson *et al.*, 1960). The difference between the phosphorus determined in this manner and that found in (a) above constitutes the ML-P. The phosphorus of this fraction is probably derived chiefly from phosphocreatine (Ennor and Rosenberg, 1952) but the phosphorus of certain other compounds would also appear here (e.g. ribose-1-phosphate and acetyl phosphate) (Lowry and Lopez, 1946) and in addition a small percentage of the phosphorus of several other compounds (e.g. adenosine triphosphate) would be determined (Weil-Malherbe and Green, 1951).

(c) *Readily acid-hydrolyzable phosphorus* (AL-P)

This was determined by the method of LePage and Umbreit (1948). This fraction is derived principally from adenosinetriphosphate (Bailey, 1949), but phosphorus from other compounds (e.g. adenosinediphosphate, hexose diphosphate, glucose-1-phosphate) would also appear here.

(d) *Acid-stable phosphorus* (AS-P) *and total acid-soluble phosphorus* (T-P)

The AS-P is the difference between the T-P of the extract determined by the method of King (1932), and the combined phosphorus of fractions (a), (b), and (c) above. This fraction includes the phosphorus of adenylic acid, inosinic acid, the AS-P of adenosine di- and triphosphate, the phosphorus of various phosphorylated sugars and possibly other compounds. Lactic acid was determined by the method of Barker and Summerson (1941).

In Table I analytical data are presented indicating the degree of agreement between different samples from the same fish and between samples from different fish. These fish were killed in an unexercised condition and at once frozen in liquid nitrogen. The data for ML-P suggest that the flounder and one trout (fish b, Table I) were perhaps not as unexercised as they were thought to be.

TABLE I. The acid-soluble phosphorus and lactic acid content of the muscle of freshly killed fish.[2]

Species	Milligrams phosphorus per gram of muscle					Lactic acid
	I-P	ML-P	AL-P	AS-P	T-P	
Rainbow trout						*mg/g muscle*
(a) Average[3]	0.82	0.46	0.47	0.52	2.27	2.38
Range	0.80–0.84	0.44–0.47	0.43–0.49	0.50–0.53	2.24–2.29	2.29–2.48
(b) Average[3]	1.36	0.05	0.43	0.57	2.41	0.78
Range	1.31–1.45	0–0.11	0.40–0.46	0.51–0.63	2.30–2.52	0.71–0.93
Starry flounder						
Average[4]	1.32	0.04	0.19	0.43	1.98	0.49
Range	1.25–1.46	0.01–0.07	0.15–0.22	0.37–0.47	1.85–2.19	0.43–0.59

[2]All fish were unexercised. The starry flounder had been in captivity about 2 months.
[3]Of four samples of lateral-dorsal muscle—fish (a) weighed 260 g, fish (b) 120 g.
[4]Of six samples of white muscle from the underside of the fish—fish weighed 320 g.

As will be seen in other results tabulated later, some variation between individual fish, previously paired to be as nearly alike as possible as to size and treatment before killing, is a difficulty we have been unable to overcome. A second limitation of the work is indicated by the data for ML-P for trout b and the flounder. Owing to the relatively large quantity of inorganic phosphorus present in the extracts, the error of the method of measurement of phosphorus introduces a variation of about ± 0.025 mg P/g of muscle into the estimation of each fraction. For this reason, beyond the fact that the quantity is very small, little meaning can be attached to any fraction if the quantity of phosphorus it contains is less than about 0.1 mg/g of flesh.

RESULTS AND DISCUSSION

Tables II and III record the results of an experiment with rainbow trout and starry flounders. To provide a clearer presentation of certain events accompanying the onset of rigor observed in this experiment and to confirm the very marked difference in the relation found between lactic acid production and rigor at 20 and 0°C, an experiment with unexercised trout was performed in which samples were taken at shorter time intervals. The results are shown in Table IV.

In general, the effects of the degree of exhaustion of the fish at death and of the storage temperature *post mortem* on the time-course of rigor, are in agreement with those previously described by others. That is, the greater the degree of exhaustion of the fish at death and the higher the storage temperature, the earlier

TABLE II. *Rigor mortis*, acid-soluble phosphorus and lactic acid in the muscle of rainbow trout as influenced by the degree of exhaustion of the fish at death and of the subsequent storage temperature. Weight of fish about 300 g each.

Time *post mortem*	Condition of fish	mg P per gram of muscle					Lactic acid
		I-P	ML-P	AL-P	AS-P	T-P	
hours							*mg/g muscle*
Stored without ice, air temperature 17 to 19°C							
0	(1) Unexercised	0.84	0.51	0.59	0.55	2.50	1.22
1	Limp	1.38	0.17	0.44	0.45	2.44	3.48
3.5	Very slight stiffening	1.27	0.26	0.60	0.38	2.51	4.82
8.5	Nearly full rigor	1.64	0.09	0.35	0.43	2.51	5.31
13.5	Full rigor	—	—	—	—	—	—
24	Softening	2.14	0.00	0.14	0.23	2.51	6.64
34.5	Soft	2.00	0.06	0.13	0.19	2.37	6.77
0	(2) Exercised	0.75	0.37	0.63	0.64	2.39	1.97
1	Limp	1.09	0.32	0.51	0.47	2.39	2.34
3.5	Very slight stiffness	1.34	0.13	0.48	0.49	2.43	3.72
8.5	Full rigor	1.88	0.12	0.06	0.30	2.36	5.64
13.5	Softening	—	—	—	—	—	—
24	Soft	1.84	0.13	0.01	0.23	2.21	5.09
34.5	Soft	1.89	0.08	0.01	0.19	2.17	5.62
0	(3) Exhausted	1.38	0.18	0.31	0.37	2.23	4.44
1	Full rigor	1.97	0.23	0.04	0.33	2.36	4.99
3.5	Full rigor	1.90	0.11	0.00	0.30	2.28	5.53
8.5	Soft	1.87	0.13	0.01	0.27	2.28	5.63
24	Soft	1.94	0.04	0.06	0.15	2.19	4.86
34.5	Soft	1.79	0.11	0.05	0.15	2.09	5.19
Stored in ice							
0	(4) Unexercised	1.06	0.22	0.61	0.51	2.40	1.48
1	Limp	1.08	0.17	0.47	0.54	2.26	1.61
3.5	Limp	1.01	0.29	0.56	0.49	2.35	1.67
8.5	Limp	1.19	0.15	0.34	0.43	2.11	2.28
14.5	Fairly stiff	1.65	0.07	0.24	0.42	2.38	3.05
23.5	Full rigor	1.61	0.12	0.07	0.36	2.16	3.59
48.5	Full rigor	1.67	0.08	0.11	0.33	2.19	4.59
58.5	Full rigor	—	—	—	—	—	—
79.5	Softening	1.46	0.12	0.08	0.30	1.95	4.17
0	(5) Exercised	1.25	0.03	0.49	0.37	2.14	3.21
1	Limp	1.39	0.05	0.37	0.42	2.23	2.74
3.5	Limp	1.37	0.07	0.28	0.40	2.12	3.42
8.5	Full rigor	1.62	0.10	0.04	0.42	2.18	4.13
14.5	Full rigor	1.79	0.03	0.01	0.43	2.25	4.50
23.5	Full rigor	1.76	0.06	0.04	0.40	2.25	4.50
48.5	Full rigor	1.62	0.09	0.05	0.30	2.01	4.89
58.5	Soft	—	—	—	—	—	—
79.5	Soft	1.57	0.00	0.00	0.26	1.83	4.07
0	(6) Exhausted	1.79	0.22	0.14	0.43	2.45	4.84
1	Full rigor	2.04	0.04	0.01	0.38	2.46	4.20
3.5	Full rigor	1.82	0.01	0.05	0.38	2.26	5.30
8.5	Full rigor	1.93	0.00	0.01	0.39	2.32	4.49
14.5	Softening	1.96	0.07	0.04	0.40	2.47	3.83
24	Soft	1.90	0.00	0.04	0.33	2.27	3.95
49	Soft	1.80	0.06	0.04	0.32	2.21	4.12
79	Soft	1.87	0.00	0.00	0.27	2.15	4.29

TABLE III. *Rigor mortis*, acid-soluble phosphorus and lactic acid in the muscle of starry flounder as influenced by the degree of exhaustion of the fish at death and of the subsequent storage temperature. Weight of fish about 300 g each.

Time *post mortem*	Condition of fish	mg P per gram of muscle					Lactic acid
		I-P	ML-P	AL-P	AS-P	T-P	
hours							*mg/g muscle*
Stored without ice, air temperature 17 to 19°C							
0	(1) Unexercised	1.11	0.17	0.23	0.37	1.87	1.08
1	Limp	1.05	0.10	0.30	0.32	1.77	1.28
3.5	Very slight stiffening	1.17	0.03	0.37	0.35	1.92	2.19
8.5	Nearly full rigor	1.35	0.11	0.18	0.27	1.91	3.25
14.5	Full rigor	1.56	0.15	0.01	0.30	2.02	3.44
24	Softening	1.61	0.09	0.04	0.20	1.95	2.55
33.5	Soft	1.62	0.04	0.06	0.19	1.92	3.44
0	(2) Exercised	1.10	0.07	0.36	0.25	1.77	0.86
1	Limp	1.38	0.10	0.35	0.29	2.12	1.08
3.5	Very slight stiffening	1.11	0.00	0.34	0.25	1.69	1.82
8.5	Full rigor	1.33	0.07	0.17	0.16	1.74	2.34
14.5	Full rigor	—	—	—	—	—	—
24.5	Soft	1.45	0.12	0.08	0.08	1.72	2.38
33.5	Soft	1.39	0.09	0.05	0.06	1.58	1.57
0	(3) Exhausted	1.20	0.07	0.20	0.24	1.71	2.15
1	Slight stiffening	1.12	0.12	0.25	0.17	1.66	1.78
3.5	Full rigor	1.33	0.10	0.14	0.21	1.79	2.67
8.5	Full rigor	1.40	0.15	0.04	0.12	1.72	3.18
14.5	Full rigor	—	—	—	—	—	—
24	Softening	1.35	0.09	0.07	0.13	1.67	2.68
33.5	Soft	1.56	0.02	0.00	0.04	1.61	2.32
Stored in ice							
0	(4) Unexercised	0.97	0.24	0.15	0.30	1.66	0.37
1	Limp	0.85	0.20	0.25	0.23	1.53	0.56
3.5	Limp	0.97	0.17	0.23	0.25	1.63	0.84
8.5	Limp	0.99	0.14	0.27	0.29	1.69	1.06
14.5	Slight stiffening	0.99	0.18	0.12	0.28	1.57	1.89
24	Full rigor	1.19	0.08	0.06	0.25	1.57	1.63
48	Full rigor	1.10	0.14	0.07	0.14	1.44	2.33
79	Softening	0.97	0.16	0.07	0.09	1.29	1.92
0	(5) Exercised	0.93	0.18	0.09	0.22	1.58	0.93
1	Limp	0.91	0.14	0.08	0.24	1.92	0.91
3.5	Stiffening	1.07	0.07	0.04	0.19	1.55	1.07
8.5	Full rigor	1.16	0.07	0.00	0.13	1.91	1.16
24	Softening slightly	1.07	0.09	0.00	0.14	1.48	1.07
48	Softening	0.62	0.08	0.02	0.07	1.30	0.62
80	Soft	0.63	0.13	0.08	0.07	1.12	0.63
0	(6) Exhausted	1.09	0.10	0.22	0.30	1.77	2.26
1	Limp	1.09	0.06	0.20	0.33	1.69	1.95
3.5	Stiffening	1.09	0.17	0.11	0.31	1.76	2.74
8.5	Full rigor	1.13	0.07	0.05	0.30	1.74	2.62
24	Full rigor	1.12	0.10	0.03	0.26	1.69	2.78
48	Soft	1.11	0.09	0.04	0.14	1.45	2.73
84	Soft	1.12	0.08	0.05	0.07	1.32	2.24

TABLE IV. The relation between onset of *rigor mortis* and changes in the molybdate-labile phosphorus, acid-labile phosphorus and lactic acid concentration in the muscle of unexercised rainbow trout stored at 0° and 20°C. Weight of fish about 300 g each.

Time *post mortem*	Condition of fish	mg P/g of muscle ML-P	mg P/g of muscle AL-P	Lactic acid
hours				*mg/g muscle*
Stored at 20°C				
0	Limp	0.27	0.45	1.17
1	Limp	0.38	0.49	1.79
2	Limp	0.48	0.44	1.82
3	Slight stiffness	0.37	0.50	3.29
6	Near full rigor	0.06	0.22	6.64
9	Full rigor	0.14	0	7.11
12	Full rigor	0.14	0.12	7.15
15	Softening slightly	0.12	0.10	7.12
18	Softening	0.14	0	7.34
21	Softening	0.11	0.10	7.05
Stored in ice				
0	Limp	0.17	0.56	1.30
1	Limp	0.24	0.50	1.56
2	Limp	0.34	0.53	1.45
3	Limp	0.42	0.48	1.49
6	Limp	0.19	0.43	1.99
9	Slight stiffness	0.08	0.25	2.34
12	Increased stiffness	0	0.23	2.68
15	Near full rigor	0.06	0.14	2.30
18	Full rigor	0	0.09	2.84
21	Full rigor	0.07	0.14	3.38

rigor sets in and the shorter time it persists (Ewart, 1887; Anderson, 1909; Leim *et al.*, 1927; Benson, 1928; Schlie, 1934; Cutting, 1939; Jul, 1952; Pavlov, 1956). In one apparent exception to this (the onset of rigor at the same time in the two exercised flounders (Table III)) the analytical data indicate that in fact the fish stored in ice was more nearly exhausted than exercised, presumably the result of low glycogen reserves in the muscle at the start of the exercise period.

In only five fish (Tables II and IV) was ML-P found in excess of 0.25 mg/g of muscle. These were all trout. Four were unexercised and one exercised. In each of the unexercised fish there was evidence of an increase in ML-P during a short period of time after killing. This increase occurred either directly to a higher value than that found in the freshly killed fish (two fish), or to a higher value than that reached after an initial decrease (two fish). It appears likely that the difference observed between these two pairs resulted from differences in the speed with which the initial sample was taken and that the usual course of events in the unexercised trout consists of a decrease in ML-P followed by a period of recovery. In each of these fish the ML-P decreased to very low values

soon after the recovery period. In the exercised fish the quantity decreased but only very slightly in the first hour *post mortem*. In all of the other fish that contained any appreciable quantity initially the concentration of ML-P decreased from the time of killing. In all of the fish, regardless of the initial course of events, the concentration of ML-P reached very low values while fairly high concentrations of AL-P remained in the muscle and before *rigor mortis* was fully established. With the exception of the initial changes observed in the unexercised fish, the relation between the ML-P concentration and rigor in trout and flounder is qualitatively like that observed by Fujimaki and Kojo (1953) in frigate mackerel and by Bendall (1951) in rabbit muscle. It should be pointed out that Fujimaki and Kojo examined their fish only at killing and again 4 and 8 hours *post mortem*. The quantity of ML-P found in a number of fish in the present work at the time they were killed was very low. The reason for this is not clear, but Tarr (1949) found similar low values in freshly killed fish, and suggested that they probably resulted from very rapid breakdown of phosphocreatine during struggling (see also Bendall, 1951). As will be seen below, in none of the commercially caught sockeye salmon that we have examined has any appreciable quantity of phosphorus been found in this fraction. These fish had all undoubtedly struggled vigorously before being landed.

In two unexercised trout (Table II) a marked decrease in AL-P was observed between killing and 1 hour *post mortem*, followed by an increase to about the same concentration as that found in the muscle at the time of killing. In the two unexercised flounders (Table III) there appeared to be a small increase in the phosphorus of this fraction during the first few hours *post mortem*. In each of these four fish, after these initial changes, the AL-P decreased to very low values. In the remainder of the fish, the acid-labile phosphorus either decreased from the time of killing, or after a short initial period during which it remained fairly constant.

In all of the fish, it is clear that the onset and development of rigor occurred during the period of time when the concentration of AL-P in the muscle was decreasing to a very low value. Full rigor did not appear to become established until the phosphorus of this fraction had nearly disappeared. This is in agreement with the previous findings of Noguchi and Yamamoto (1955a, b) who studied the *post-mortem* changes in the contractability of fish muscle (in response to perfusion with water) in relation to the amount of AL-P the muscle contained.

It is interesting that the range of concentration of AL-P over which rigor became established in the fish is similar to that (about 0.4 mg/g and lower) over which it became established in rabbit psoas muscle (Bate-Smith and Bendall, 1947; Bendall, 1951) and in whale muscle (Marsh, 1952). There was a tendency for the onset of rigor to occur at higher concentrations of AL-P in fish muscle at 20°C than at 0°C. It appears from this that a similar relation between the

critical level of adenosinetriphosphate (for onset of rigor), temperature, and pH may exist for fish as was found for rabbit muscle by Bendall (1951). However, the present data are insufficient to establish this.

The phosphorus of the acid-stable fraction decreased *post mortem*, but unlike that of the molybdate-labile and acid-labile fractions, did not fall rapidly to low values during the onset of rigor, but rather continued to decrease at a fairly steady rate during rigor. By the time rigor was resolved, the phosphorus of this fraction in trout had decreased to a value of about 0.3 mg/g of muscle or less, while in flounder it was generally down to 0.2 mg/g or less.

In unexercised fish (Tables II to IV) it will be seen that there was a different relation between the course of formation of lactic acid and the onset of rigor at 0°C and at room temperature. At the higher temperature, the concentration of lactic acid in the muscle reached its maximum value at about the time full rigor was established, whereas at 0°C full rigor was established while lactic acid was still being actively formed and had reached only about 50% of its final maximum concentration. In exhausted fish, on the other hand, at either temperature the maximum concentration of lactic acid was reached either by the time full rigor was established or very soon thereafter. In exercised fish, as would be expected, the course of events tended to be intermediate between these two extremes.

Although it has long been known that the occurrence of rigor is not dependent on lactic acid formation (Hoet and Marks, 1926) the available data indicate that there is a relation between the duration of rigor in fish and the quantity of lactic acid accumulated in the muscle *post mortem*. That is to say, the greater the concentration of lactic acid reached (or the lower the pH of the muscle) the longer would rigor endure (Reay and Shewan, 1949). In the present work, while there is some evidence for such a relation in the fish stored at room temperature, a different relation is evident in those stored in ice. In these fish it appears that the longer lactic acid continues to be produced *post mortem*, the longer is the time before rigor is resolved. This relation can also be seen in the data for the fish stored at room temperature. A portion, but by no means all, of the increase in time results from delay in onset. Although, as previously thought, the establishment of a low pH in fish muscle may be a factor in prolonging the duration of rigor, it does not seem to be unreasonable to suggest that for rigor to persist once it has been established, it may also be necessary that an energy-yielding process be proceeding in the muscle and that once this process has ceased, or decreased to a very low rate, then rigor begins to disappear. It is interesting to recall Ewart's remark made in 1887: "If before the rigor appears the latent energy of the muscles has all but exhausted . . . the rigor though well marked will be of short duration, while on the other hand, if a considerable amount of rigor producing material [unspecified] is left when the stiffening supervenes, the rigor, though not strikingly resembling a tetanic spasm will be more intense and more persistent."

A number of sockeye salmon were caught under commercial conditions and examined in the same fashion as were the trout and flounders. The data obtained (Tables V to VII) are quite similar to those recorded above for exercised or

TABLE V. *Rigor mortis*, acid-soluble phosphorus and lactic acid in the muscle of gill-net caught sockeye salmon as affected by the condition of the fish at killing and the storage temperature.[5]

Time *post mortem*	Condition of fish	mg P per gram of muscle				Lactic acid	Time to	
		I-P	ML-P	AL-P	AS-P		Onset of rigor	End of full rigor
hours						*mg/g muscle*	*hours*	*hours*
Stored without ice[6]								
0	(1) Lively, killed on landing	1.37	0.00	0.34	0.38	5.94	1	6
4	Full rigor	1.89	0.04	0.04	0.25	8.53		
16	Soft	1.95	0.00	0.00	0.11	8.77		
0	(2) Exhausted, killed on landing	1.49	0.07	0.28	0.46	5.90	1	5
3	Full rigor	1.82	0.06	0.06	0.38	7.32		
16	Soft	1.83	0.17	0.03	0.13	7.84		
0	(3) Allowed to die in air	1.22	0.18	0.30	0.59	6.14	1	3
3	Full rigor	1.66	0.15	0.09	0.34	9.24		
16	Soft	1.70	0.19	0.06	0.35	8.31		
0	(4) Dead on landing	1.27	0.06	0.28	0.50	6.12	<1	4
3	Full rigor	1.53	0.15	0.02	0.41	8.64		
17	Soft	1.59	0.04	0.07	0.33	8.65		
Stored in ice								
0	(5) Like (1)	1.07	0.09	0.57	0.41	—	4	17
1	Limp	1.05	0.12	0.53	0.36	4.05		
4	Limp	1.36	0.04	0.37	0.39	6.05		
13	Full rigor	1.51	0.10	0.00	0.36	7.97		
45	Softening	1.52	0.19	0.00	0.34	8.25		
72	Soft	1.52	0.00	0.00	0.24	5.53		
0	(6) Like (2)	1.15	0.04	0.36	0.49	5.61	1	17
1	Limp	1.30	0.00	0.19	0.40	6.70		
3	Full rigor	1.34	0.00	0.09	0.33	6.44		
13	Full rigor	1.46	0.05	0.04	0.34	7.96		
45	Softening	1.67	0.00	0.00	0.38	8.74		
72	Soft	1.57	0.00	0.00	0.36	8.38		
0	(7) Like (3)	1.36	0.09	0.28	0.43	5.76	<1	15
3	Full rigor	1.57	0.07	0.02	0.50	7.57		
23	Softening	1.66	0.00	0.04	0.41	7.84		
48	Soft	1.71	0.00	0.03	0.36	6.41		
0	(8) Like (4)	1.31	0.00	0.41	0.36	4.76	1	15
3	Full rigor	1.58	0.03	0.00	0.44	6.56		
23	Softening	1.62	0.01	0.00	0.37	7.49		
48	Soft	1.47	0.08	0.07	0.35	6.96		

[5]These fish weighed about 6 lbs each and were caught in the mouth of the Fraser River near Steveston, B.C., July 26–27, 1960. The water temperature was 18°C.

[6]The storage temperature for the fish stored without ice was 24°C at the start and decreased to about 20°C at the end of the experiment.

TABLE VI. Acid-soluble phosphorus and lactic acid in the muscle at the time of landing and the course of *rigor mortis* in sockeye salmon from a single gill net set.[7]

Appearance of fish at landing	mg P per gram of muscle				Lactic acid	Time to	
	I-P	ML-P	AL-P	AS-P		Onset of rigor	End of full rigor
					mg/g muscle	*hours*	*hours*
Fairly lively	1.02	0.0	0.62	0.49	5.87	1	5
" "	0.97	0.14	0.54	0.49	5.42	1	5
Exhausted	1.09	0.10	0.43	0.55	5.95	1	3
"	1.04	0.17	0.35	0.54	6.26	1	3
"	1.09	0.17	0.32	0.55	6.09	1	5
"	0.99	0.12	0.35	0.60	5.46	1	4
"	1.11	0.19	0.43	0.61	5.96	1	4
Dead	1.16	0.17	0.30	0.48	6.26	<1	4

[7]These fish, each weighing about 6 lb, were taken from the Fraser River near Albion, B.C., July 31, 1960. The fish were swimming upstream against a strong current. They were selected from various points along the length of the net and were killed by a blow on the head and at once sampled as they were landed. The set lasted 30 minutes. For observation of *rigor mortis* they were stored without ice at a temperature of 19°C. All fish were judged to be completely out of rigor 14 hours *post mortem*.

exhausted trout. A somewhat higher maximum concentration of lactic acid was found to accumulate in the salmon than in the trout. The very marked influence of icing in prolonging the duration of rigor is obvious and as in the trout appears to be related to a reduction in the rate of glycolysis. It is also evident that even in these commercially caught fish there is a large production of lactic acid *post mortem*. The salmon weighed about 6 pounds each compared to about ½ pound for trout and would therefore be expected to cool more slowly in ice; consequently, it is likely that a more efficient system of chilling could lengthen the duration of rigor in salmon.

Under conditions of elevated summer temperatures it is clear that if processing cannot be carried out within a very short time of catching, icing would be most beneficial. It should be pointed out that to derive the greatest benefit from icing it must be carried out as quickly as possible after the fish are landed in order to take the fullest advantage of the energy reserves of the muscle. The data for the salmon (particularly Table VI) indicate that at landing they are remarkably uniform but such differences as are seen, and also the noticeable effect of allowing the fish to die in air after landing, rather than killing them at once, suggest that important increases in duration of rigor in salmon could be effected if a method of catching were used that would greatly shorten the period from first contact to killing. Although, as suggested by Reay and Shewan (1949), this possibility may be only of academic interest now, it exists and should not be overlooked.

TABLE VII. *Rigor mortis*, acid-soluble phosphorus and lactic acid in seine-caught sockeye salmon as affected by the condition of the fish at killing and the storage temperature.[8]

Time *post mortem*	Condition of fish	Mg P per gram of muscle I-P	ML-P	AL-P	AS-P	Lactic acid	Time to Onset of rigor	Time to End of full rigor
hours						*mg/g muscle*	*hours*	*hours*
Stored without ice[9]								
0	(1) Killed on landing	1.18	0.17	0.34	0.53	5.57	1	3*
3	Full rigor	1.40	0.11	0.0	0.43	8.73		
19	Soft	1.83	0.0	0.0	0.26	9.96		
0	(2) Killed 4 minutes after landing	1.23	0.11	0.48	0.42	5.96	1	2*
3	Full rigor	1.76	0.08	0.02	0.40	9.82		
19	Soft	1.70	0.11	0.06	0.33	9.86		
0	(3) Died in air, 15 minutes after landing	1.45	0.05	0.20	0.43	6.55	<1	1*
3	Full rigor	1.70	0.0	0.03	0.35	9.95		
19	Soft	1.86	0.0	0.07	0.35	9.76		
Stored in ice								
0	(4) Like (1)	0.96	0.19	0.50	0.47	4.95	3	16**
3	Stiffening	1.19	0.06	0.38	0.46	4.46		
6	Full rigor	1.55	0.01	0.06	0.55	5.81		
19	Full rigor	1.74	0.0	0.01	0.49	6.57		
43	Softening—firmer than (5) and (6)	1.86	0.0	0.06	0.40	7.14		
0	(5) Like (2)	1.19	0.11	0.46	0.53	5.16	1	18**
2.5	Near full rigor	1.23	0.18	0.18	0.46	6.27		
6	Full rigor	1.31	0.13	0.04	0.48	6.36		
19	Full rigor	1.61	0.16	0.03	0.48	7.35		
43	Softening	1.69	0.15	0.0	0.49	7.28		
0	(6) Like (3)	0.90	0.18	0.50	0.50	5.66	<1	18**
2	Near full rigor	1.38	0.07	0.25	0.36	5.59		
6	Full rigor	1.47	0.08	0.06	0.44	7.02		
19	Full rigor	1.64	0.0	0.08	0.39	7.74		
43	Softening	2.10	0.08	0.05	0.32	8.10		

[8]These fish were caught at sea near Pt. Roberts, Washington, U.S.A., Aug. 10, 1960.

[9]Air temperature increased from 25°C to 28°C in 3 hours *post mortem*, then decreased to 23°C at 19 hours.

*These fish were all judged to be completely out of rigor 6 hours *post mortem*, with the order of firmness at that time being 1>3>2.

**Unfortunately observations of these three fish were not made between 19 and 43 hours *post mortem*, consequently the duration of full rigor is not known and the times given are minimum values.

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Note added in proof

Since this paper was submitted for publication, a further paper by N. R. Jones and J. Murray has appeared (Zeitschrift für vergleichende Physiologie, *44*: 174–183 (1961)) in which they have described the relation between *rigor mortis* and changes in the concentrations of nucleotides in codling (*Gadus callarias*) muscle at 0°C.

In contrast to our findings with unexercised trout and flounders, in which the concentration of acid-labile P was maintained for several hours *post mortem* at 0°C, Jones and Murray found that there was no observable maintenance of the adenosinetriphosphate level in codling muscle under the same conditions. This difference may have arisen as a result of species differences or of nutritional differences between the fish used in the two investigations. Jones and Murray compared their results with the known maintenance of adenosinetriphosphate for a period of time *post mortem* in mammalian muscle, and suggested the difference resulted from the very low concentration of glycogen in fish muscle. Our results indicate that at least in certain fish the difference between mammalian and fish muscle is not so great as to preclude the maintenance of adenosinetriphosphate for some time *post mortem* in the latter.

A point of interest that arises from a consideration of the results recorded in these and other papers is the fact that pre-rigor fish even of the same species are not necessarily uniform biochemically and can have quite different concentrations of acid-soluble phosphorus compounds and lactic acid in their muscles depending on their previous history. This fact should be taken into account in investigations dealing with pre-rigor freezing of fish.

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Variability in Aerial Counts of Spawning Salmon[1,2]

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ABSTRACT

A study of spawning ground surveys for pink salmon (*Oncorhynchus gorbuscha*) was made in two streams on Kodiak Island. An experimental design is described which permits replication of observers' counts of spawning salmon. The variance in an observer's estimate was found to be proportionate to the size of the estimate. The experiments indicated that an observer will detect differences in population size of plus or minus 50%. The relationship between counts of one observer and another changes within different streams, but within each river the observations of one observer were correlated with those of another. The results of the experiments are summarized in recommendations for aerial surveys of spawning salmon.

INTRODUCTION

Each summer in Alaska salmon return to spawn and die in their native streams. The abundance of spawners in the streams and the number taken by fishermen affect the health of the runs. Counts of abundance of spawners are therefore important to both industry and regulatory agencies. During fishing seasons the fishery is often opened or closed depending on current estimate of the size of escapement.

Runs to some of the more important streams are counted through weir fences or past observation towers. This is the case for the sockeye or red salmon (*Oncorhynchus nerka*) of Bristol Bay. There the bulk of the run enters into half a dozen rivers where the salmon may be enumerated in the trunk stream before dispersal to many tributaries.

The situation is different with two other species, the pink and chum salmon (*O. gorbuscha* and *O. keta*), in other parts of Alaska. Pinks and chums spawn in about 2000 rivers, large and small, spread over an extended coastline. The variability is great between streams and the number of streams that must be enumerated is large. It is not economically feasible to make direct counts with weirs or towers in every stream. The abundance of spawners in several hundred streams is estimated by visual observations from airplanes.

It is obvious that direct counts cannot be made of every fish in a stream while the observer flies overhead at 70 to 100 miles an hour. Spawning populations often number up to several hundred thousand and may occupy only a few miles of river with several spawners to a square yard. We must rely on an estimate of some sort. The method commonly used is to count by hundreds

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or by thousands. The number of fish required to fill the counting unit of 100 or 1000 must be estimated. The number of these estimates multiplied by the basic counting unit supplies the total count. This paper is concerned with measuring the variability of this type of estimation.

The problems of measuring the accuracy or precision of escapement enumeration have received little attention in fisheries literature. Atkinson (1944) was one of the first to consider the problem. He recognized that the spawners were not a stationary population and that new entrants arrived to take the place of those that died after spawning. Thus any estimate made at one time will not correctly estimate the total number of spawners. With a knowledge of the length of life of the individuals and by conducting several surveys it is possible to correct for this type of error (Gangmark and Fulton, 1952).

The changing population is not the only reason why an individual count does not provide the total number of spawners. Many fish remain unobserved in deep pools, under overhanging brush or trees, or below the limits of visibility in turbid glacier-fed rivers.

Observations will give at best an index, an unknown portion, of the number of spawners, even if corrected for the changing population. In practice it is more convenient to determine the maximum rather than the average observed population. Assuming that the length of life of individuals on the spawning ground is relatively constant from year to year we can use the maximum observed abundance as an index to the number of spawners.

It is the variability in this index that will be the subject of our discussion. We will limit ourselves to the variation in the index that measures peak abundance and will not consider the differences between the index and the true population.

We are dealing with the ability of human beings to estimate numbers. Unfortunately this seems to be a field that has been neglected by psychologists. The only studies that have been made deal with the ability to perceive a small number of objects or the time period required to differentiate between groups of different numbers. These studies have little application to our problem.

We are evaluating a perceptual matter and doing so in a subjective manner. In most cases where physical quantities or properties are very complex or poorly understood we turn to a subjective method of evaluation. Measures of taste flavors or of personal comfort are examples. Advocates of subjective measurements usually argue for them on the basis of impracticability of physical measurements for the purposes intended. The difficulties usually recognized are that the evaluation will suffer variability between and within observers. Less often the difficulties in training of observers are discussed.

The subjective method will be usable if we do not require a high degree of accuracy. In fact, in surveys to be discussed each single judgment need not be correct. Hopkinson (1953) has pointed out that the mean of a convenient number of judgments should not drift and that the variance of the judgments should be small in relation to the differences in which the experiment is interested.

Thompson[3] discussed spawning survey estimates based on the completely subjective ratings of *good*, *fair* or *poor*, accompanied by numerical estimates. He found that often escapements rated by one observer as poor were associated with higher numerical counts than estimates rated good by another observer. He questioned the comparability of different observers and suggested that standardized methods be applied.

The problem of estimating large numbers was considered by Rae (1952) in estimating the numbers of plankton in a microscope transect. He found that estimates were consistent, with little difference between observers.

In this paper our objectives are to determine the precision of the spawning survey indices and to determine some of the factors which affect them by replicating the observations.

METHODS

The study was made in two streams on Kodiak Island (at Uyak and Deadman Bays). These streams are of moderate size and have had peak spawning counts of pink salmon between 10,000 and 150,000 in the past 10 years. The physical characteristics of the streams are similar to many other salmon spawning rivers in Alaska (Fig. 1). The variability due to weather conditions and water turbidity was minimized by conducting all observations during good weather and during normal stream flows.

In order to avoid personal bias, the earlier estimates of an observer should not be allowed to prejudice his subsequent estimates of the same group of fish. This was avoided by having the observer make his counts in such a manner that he was not aware of the total he was reporting.

Each stream was divided into two sections which included the total spawning area of the stream. Counts were made of the total stream as well as each section. The counts were made by estimating groups of 100's and 1000's while flying both upstream and downstream. Thus each section and the total stream were counted four times. Prior to each flight the order in which the sections were to be counted was determined by lot, with the restriction that no section would be counted twice in a row by the same counting unit. This meant that after a particular section of stream was counted either a new section or another counting unit was presented.

For further insurance that the counts would not be remembered, the number of estimated basic units was registered on a hand counter in the following manner. The pilot recorded the section being counted, direction of flight, and a number that he set on the dials of the hand counter. He then handed the counter to the observer after sealing the dials with a piece of opaque tape. The observer recorded the number of estimates of the counting unit immediately as they were sighted by pressing the lever on the counter. When the section was completed

[3]Thompson, W. F. 1948. Memorandum for stream surveys for scientific personnel. Fisheries Research Institute, University of Washington; unpublished.

FIG. 1. Deadman River, Kodiak Island. Photo by Ron Lopp. Section C of the stream extends from the mouth up to the point "P". Section D extends from "P" to "Q".

the observer recorded the total appearing on the dials and reset the counter to zero before returning it to the pilot. The actual count was obtained later by subtracting the preset number from the final number recorded by the observer. This assured that neither the pilot nor observer knew the count recorded for any section.

It was not feasible to randomize the use of airplanes because of the time and expense required in landing and taking off again. Each observer completed a series of observations duplicating each section and the total of the stream and then changed airplanes.

The sections to be counted were randomized only within each stream to minimize the travel between streams. A program was completed in a stream before starting in the second stream. The same flight plan was used by each airplane and an attempt was made to make the observations at as near the same time as possible.

All counts were made from small, slow-flying airplanes. The type of airplane was similar to one commonly used for crop dusting with the exception of the addition of floats for water landings. The air speed was usually kept at about 75 miles an hour (120 km/hr). The flight altitude during observation was generally about 200 feet (33 m), but varied between 100 and 300 feet depending upon the terrain.

The variables in the experiments were observers, airplanes, methods of counting (100's or 1000's), streams, and whether counts were by total stream or by sums of sections. Three experiments were run in 1957, and another experiment was conducted over a 3-year period beginning in 1956. The estimates from each experiment are tabulated in the appendix tables.

Each observer had at least one season's experience with stream surveys and all were currently engaged in making surveys for research or management purposes at the time the experiments were undertaken.

Analysis of variance procedures were used to test hypotheses of differences between the means of the variables involved. Since the comparisons are between counts, it might seem that chi-square techniques should be used to handle enumerated observations. The counts are complicated, however, by the presence of additional variability. Although the number of fish in the stream at a particular time may follow some specified distribution, different groups of fish will have different probabilities of being counted because of variation in the ability of the observer to estimate the basic counting unit. The result of these two variations can be thought of as a measured variable rather than a precise count. Therefore, analysis of variance is applied. The original data were transformed to logarithms to correct for a proportional relationship between the means and the variances. Figure 2 shows the relationship between the means and the variances.

In applying the results of the analysis of variance we should like to consider that our primary effects are drawn randomly from a larger population. We would consider that our model of analysis was either random or possibly mixed

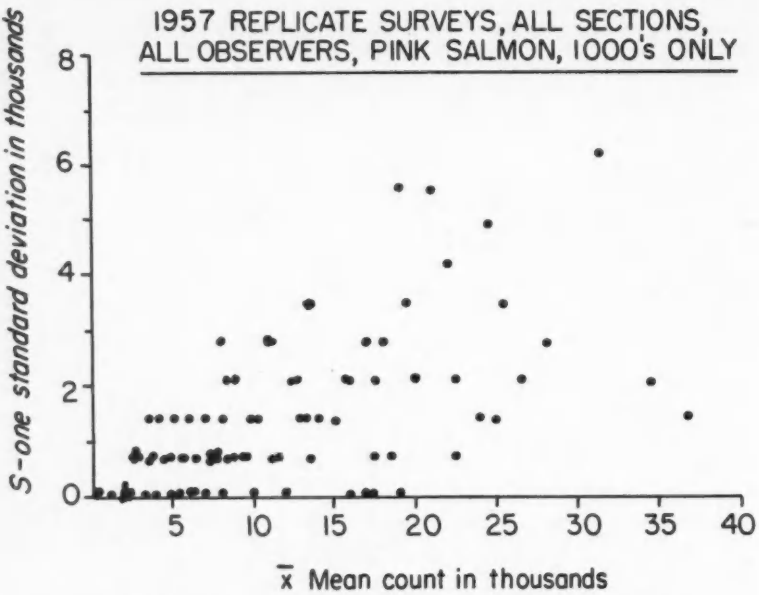

FIG. 2. Comparison of observers' mean count and standard deviation.

if we wished to consider only a particular series of streams or only two possible ways of making the counts. However, it was not feasible to obtain enough degrees of freedom for any of the main effects to have any sensitivity in a mixed model. Either we could not collect enough observers together or the time element prohibited covering a large series of streams under uniform observation conditions. Therefore we have considered a series of tests on fixed models and will make some inferences from these that may apply to other situations if the individual tests have some agreement. The difficulty is recognized of drawing conclusions about the significance of main effects when interaction is present.

RESULTS FROM THE THREE EXPERIMENTS IN 1957

The analyses of data from the 1957 experiments are summarized in Table I. A consistency in the three experiments is shown. Under main effects, significant differences were found between the means of observers and between counting methods in each of the experiments. Differences between counts from the total stream or from summing the sections were not significant in all experiments. Counts between streams were significantly different in both experiments where two streams were surveyed. In the two experiments in which more than one airplane was used there was no difference between mean counts from different airplanes.

TABLE I. Summary of analysis of variance tables—1957 experiments. (MS = mean square; df = degrees of freedom; ** = $P<0.01$).

Source of variation	1st experiment Observers A & C		2nd experiment Observers A, B & C		3rd experiment Observers A, D & E	
	MS	df	MS	df	MS	df
Main effects						
Observers (O)	0.0541**	1	0.0802**	2	0.1398**	2
Airplanes (A)	0.0077	1	0.0022	1	—	–
Counting methods (C)	0.3249**	1	0.1291**	1	0.4485**	1
Total counts vs section counts (T)	0.0138	1	0.00002	1	0.0091	1
Streams (S)	2.8900**	1	—	–	0.3434**	1
Interactions						
O × A	0.0563**	1	0.0023	2	—	–
O × C	0.1296**	1	0.0401**	2	0.1638**	2
O × T	0.0095	1	0.00004	2	0.0037	2
O × S	0.0049	1	—	–	0.0026	2
A × C	0.0020	1	0.0010	1	—	–
A × T	0.0005	1	0.0068	1	—	–
A × S	0.2652**	1	—	–	—	–
C × T	0.0004	1	0.0083	1	0.0002	1
C × S	0.0060	1	—	–	0.0902**	1
T × S	0.0009	1	—	–	0.0007	1
O × A × S	0.0381**	1	—	–	—	–
O × C × S	0.0027	1	—	–	0.0844**	2
Other 3-way and higher interactions	0.0036	14	0.0044	9	0.0024	7
Within	0.0020	32	0.0038	24	0.0068	24
Total		63		47		47

Results from the tests on the two-way and higher interactions were also similar in each of the three experiments. The observer-count interaction was the only interaction present in all experiments. Apparently there was a difference between observers in their ability to count by 100's or 1000's.

A three-way interaction (observer-airplane-stream) and two-way interactions (observer-airplane and airplane-stream) were present in the first experiment. These interactions were present only in the first experiment, and a check of the field notes shows that this was correlated with and probably the result of one of the observers becoming airsick in a specific airplane over one of the streams.

In the third experiment three-way observer-count-stream and two-way count-stream interactions occur. This is associated with a low numerical value for the observations in one of the streams and indicates that the ability to count by 100's or 1000's will differ depending upon the magnitude of the count. This suggests that further studies might place some limit on the numbers where estimates by 100's and 1000's may be combined.

All other two-way, three-way and higher order interactions were non-significant.

RESULTS FROM THE THREE-YEAR EXPERIMENT

Results of the analysis of variance of the experimental data from two observers over a 3-year period are shown in Table II. The main effects supply us with little additional information. Differences in observer and stream means agree with our results from the three experiments previously discussed. The difference between years, if not intuitively obvious, must be assumed to supply a justification for the existence of any survey program. Actually we know that streams vary considerably from year to year. Some even have runs only in alternative years.

TABLE II. Analysis of variance table of pink salmon replicate surveys in 1956, 1957 and 1959, on Uyak and Deadman Rivers. Observers were A and E; counts were by 1000's. (SS = sum of squares; df = degrees of freedom; MS = mean square; ** = $P<0.01$).

Source	SS	df	MS	P
Observers (O)	0.1512	1	0.1512	**
Streams (S)	0.0398	1	0.0398	**
Years (Y)	1.0224	2	0.5112	**
Interactions				
O × S	0.2324	1	0.2324	**
O × Y	0.1010	2	0.0505	**
S × Y	0.5966	2	0.2983	**
O × S × Y	0.0241	2	0.01204	—
Within	0.1354	36	0.00376	—
Total	2,3029	47		

The main interest comes from the interactions. The presence of an observer-year interaction suggests that any difference between these two observers changes from year to year. This means that it will be difficult to determine any relationship between observers that might be used to adjust the observations to a common basis.

The observer-stream interaction indicates similar problems in trying to adjust observer differences between streams since a different factor may be necessary in different streams.

AMOUNT OF VARIABILITY

So far we have confined our discussion to the various effects that are associated with observed variations. The amount of variation is also of importance. Even though considerable variation exists, the results will be usable for purposes of an index if the variation in observation is small in comparison with changes in the abundance of fish. Actual total counts of pink salmon through weirs have shown cyclic differences as much as 800 times (5000 to 4,000,000) and

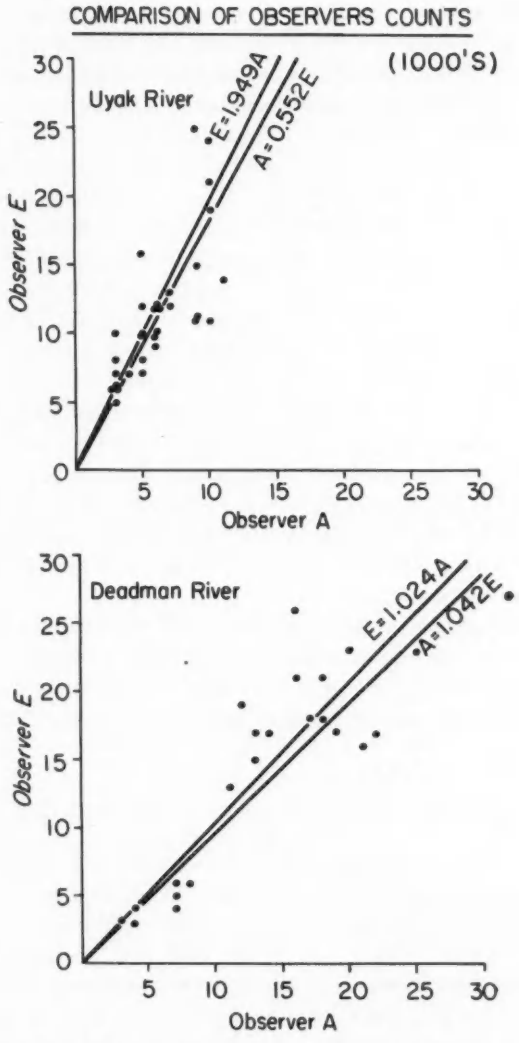

FIG. 3. Comparison of observers' counts in thousands.

variations of 10 to 15 times are very common (Rounsefell, 1958; Counts at the Snake Creek Weir[4]).

Figure 2 shows the relationship between the standard deviation computed from replicate surveys by the observers and the mean count obtained. All counts were made by 1000's and two replicates were obtained. A similar distribution was obtained by using the counts by 100's but with slightly larger standard deviations.

The limitations imposed by two replicates and the small number of observers tested should be kept in mind. The data in Fig. 2 indicate that one standard deviation of an observer's counts will not be more than $\pm 50\%$ around his mean observation. He might expect that usually it will be less than 25%. This would mean a standard deviation of 5000 for a mean count of 20,000. If we consider two standard deviations around the observer's mean we can conclude that generally speaking an observer will detect differences of $\pm 50\%$.

The magnitude of the observer-stream interaction from the 3-year experiment is shown in Fig. 3. Here the counts of the observers are paired by associating estimates made as close together as possible with respect to time. The observations by one observer are correlated with those of another in each river (Uyak, $r = 0.75$; Deadman, $r = 0.76$). However, in the case of Uyak River, there is a considerable bias between the observers, with Observer E consistently obtaining higher estimates. This is not so in Deadman River, where the regression coefficients do not differ significantly from 1.

The use of Snedecor's (1956) regression model 1A explains the fact that the coefficients of x on y and y on x are both greater than 1 for the Deadman River experiments. This model was appropriate since the variance was proportional to the counts and we assumed that the regression line passed through the origin.

RECOMMENDATIONS FOR AERIAL SURVEYS

Any use of aerial surveys should be done with a realization that the counts have value as an index and not a total count. Any calculation or compilation of survey data should be that appropriate for handling indices.

Even though stream survey counts are used only as indices, they should not be used to indicate differences of less than 50% without a careful evaluation of the variance of the observer's estimates. Only one observer should be used. If it is necessary to change observers, any factor to correct observer differences may vary from stream to stream and from year to year. Only one counting unit should be used. Substitution of different aircraft of the same type or of different experienced pilots should make little difference in the estimates obtained.

[4]Official Records of the United States Fish and Wildlife Service Snake Creek Weir, Olive Cove, Southeastern Alaska.

ACKNOWLEDGMENTS

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APPENDIX. Stream survey counts of pink salmon in Deadman and Uyak Rivers, Kodiak Island, Alaska. All figures tabulated are estimated numbers of pink salmon, in thousands.

TABLE A. Experiment No. 1, Aug. 19, 1957.

	Observer A				Observer C			
Airplane No.	7162		4590		7162		4590	
Counting unit	100	1000	100	1000	100	1000	100	1000
Stream section								
A + B	27.8	24	16.4	21	25.8	40	18.5	32
	26.0	32	17.5	19	24.6	43	14.8	26
AB	27.1	30	17.7	25	26.4	38	20.9	36
	29.1	26	17.7	19	28.2	36	17.9	27
C + D	8.1	10	8.3	10	6.0	9	7.6	17
	8.4	10	9.3	9	5.1	8	8.3	17
CD	8.8	9	8.6	10	5.2	14	10.6	19
	8.0	10	7.9	9	6.1	12	11.3	15

TABLE B. Experiment No. 2, Aug. 22, 1957.

	Observer A				Observer C				Observer B			
Airplane No.	7162		4590		7162		4590		7162		4590	
Counting unit	100	1000	100	1000	100	1000	100	1000	100	1000	100	1000
Stream section												
A + B	18.7	19	17.7	20	16.2	20	14.6	24	13.7	14	11.0	12
	20.2	20	19.2	19	16.0	29	15.0	22	16.2	19	14.8	16
AB	19.0	17	19.1	19	15.6	28	13.2	21	8.0	17	14.3	23
	20.8	22	19.6	19	14.8	25	15.4	24	12.7	17	13.2	15

TABLE C. Experiment No. 3, Aug. 29, 1957.

	Observer A		Observer D		Observer E	
Counting unit	100	1000	100	1000	100	1000
Stream section						
A + B	12.4	9	2.9	15	11.2	17
	11.7	10	5.0	11	5.3	18
AB	12.9	11	3.8	14	5.9	17
	10.0	9	3.9	11	5.3	14
C + D	6.9	8	4.1	6	4.7	10
	7.0	7	5.3	4	5.2	7
CD	7.2	7	4.6	6	4.5	11
	7.4	7	5.4	5	4.9	9

TABLE D. Estimated number of pink salmon on August 29, near the peak of the run (in thousands).

	Observer A		Observer D	
Year	Deadman River	Uyak River	Deadman River	Uyak River
1956	25	10	23	21
	32	10	27	24
	20	9	23	25
	22	10	17	19
1957	8	11	6	14
	7	9	4	11
	7	9	6	15
	7	10	5	11
1958	14	7	17	12
	13	5	15	10
	12	6	19	12
	11	6	13	12

Preparation of Deoxynucleosides, Purine and Pyrimidine Bases and Deoxyribose 1-Phosphate from Deoxyribonucleic Acid Employing Salmon Enzyme Systems[1]

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ABSTRACT

A crude enzyme system made by treating aqueous extracts of salmon kidney with protamine solution, followed by centrifugation to remove precipitated ribonucleic acid, was used to hydrolyze salmon deoxyribonucleic acid. When such hydrolyses were conducted in presence of toluene to minimize bacterial action, the deoxynucleosides of thymine, uracil, guanine and hypoxanthine were isolated in comparatively pure state. However, the digests also contained a significant proportion of unidentified non-nucleotide ultraviolet-absorbing substances. Digestions carried out in absence of toluene, with accompanying bacterial activity, resulted in formation of thymine, uracil, hypoxanthine and xanthine, which were isolated and characterized. A crude nucleoside phosphorylase enzyme was prepared from salmon livers by a method similar to that used with the kidney enzyme system. In presence of 0.2*M* KF it exhibited the following order of specificity: deoxyuridine > thymidine > deoxyguanosine > deoxycytidine > deoxyinosine > deoxyadenosine > uridine > cytidine > adenosine > inosine > guanosine > xanthosine. This preparation also exhibited marked phosphodeoxyribomutase and phosphoribomutase activities. Dicyclohexylammonium deoxyribose 1-phosphate was isolated in an amorphous form (purity 74%) from a reaction mixture containing thymidine, orthophosphate and the liver enzyme.

INTRODUCTION

In recent years fish muscle enzymes concerned with nucleic acid degradation have been studied extensively at this Station (Tarr, 1955; Tomlinson, 1958, 1959; Tomlinson and Creelman, 1960; Tomlinson and Warren, 1960; Tomlinson, Creelman and Reid, 1960), but information concerning the occurrence of such systems in other fish organs appears to be lacking. Consequently it was thought desirable to carry out a general study of the hydrolysis of deoxyribonucleic acid (DNA) by crude enzymes of certain salmon visceral organs, and of the phosphorylation of the deoxynucleosides derived therefrom to deoxyribose 1-phosphate.

MATERIAL AND METHODS

Purified, chromatographically homogeneous nucleosides, deoxynucleosides, purines and pyrimidines were purchased from the California Corporation for Biochemical Research, Los Angeles, (A grades). Dicyclohexylammonium salts of α-D-ribofuranose 1-phosphate (R1-P) and 2-deoxy-D-ribofuranose 1-phosphate (DR1-P) were analytically pure materials previously prepared in this laboratory (Tarr, 1958) which had been stored in vacuo at −20 to −30°C. The barium salts of α-D-ribofuranose 5-phosphate (R5-P) and 2-deoxyribose-D-ribofuranose

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5-phosphate (DR5-P) were prepared by known methods previously referred to (Tarr, 1959). D-ribose was purchased from the Nutritional Biochemicals Corporation, Cleveland, and 2-D-deoxyribose from the California Corporation for Biochemical Research. Dr Bernard Malin, Eli Lilly Inc., Indianapolis, kindly donated an ample supply of pure protamine sulphate.

Ribose was determined by the orcinol method (Mejbaum, 1939), deoxyribose by the Dische diphenylamine reaction as modified by Racker (1952) or by the Stumpf (1947) modification of the cysteine–H_2SO_4 acid reaction and total phosphorus by the method of Gomori (1942) after digestion with H_2SO_4 (Umbreit *et al.*, 1949). Orthophosphate, in presence of very acid labile DR1-P, was determined by the method of Friedkin (1950). The quantitative biuret reaction (Kingsley, 1939) was used to determine protein.

Kidneys, livers and ripe milts were removed from large red or white spring salmon (*Oncorhynchus tshawytscha*) or sockeye salmon (*O. nerka*) shortly after death and were promptly frozen in polyethylene bags in crushed CO_2 ice. The tissues were stored for several months at –30°C without noticeable loss of activity. However, it was found that kidneys obtained from non-eviscerated salmon which had been iced for a day or two retained only a small proportion of their ability to provide enzyme preparations capable of hydrolyzing DNA. Unless otherwise stated all procedures for isolation of the enzyme preparations used were carried out between 0 and 3°C.

EXPERIMENTAL

Preparation of DNA

Since highly polymerized DNA was not required, the method of Jones (1920) for preparation of "thymus nucleic acid" (now known to be thymus DNA) was used with minor modifications as follows:

One kilogram of finely minced ripe milts (spring, sockeye, as well as chum salmon, *O. keta*, have all yielded successful results) was stirred into 2 l of H_2O containing 100 g sodium acetate and 33 g of NaOH, and the mixture heated for 2 hr on a boiling water bath with occasional stirring. The solution was centrifuged in stainless steel cups at 9500 *g* while still hot. Since the DNA tended to gel during centrifuging, the cups were immersed in a boiling water bath for a short time prior to decanting the supernate from the insoluble residue. The pale brown viscous liquid was adjusted, while still hot, to pH about 6.5 with 50% acetic acid. The solution was then poured slowly, with very rapid stirring, into 4 l of EtOH (>99%). After standing overnight at 0°C the aqueous EtOH layer was carefully decanted, and the product dried, first by grinding with EtOH, and finally in vacuo over H_2SO_4 and solid KOH. The crude DNA was then dissolved in 300 ml of hot H_2O containing 2 g of NaOH and heated for 1 hr on a boiling H_2O bath. The suspended material was removed by filtration with suction through Whatman No. 1 paper on a steam-heated Büchner funnel and the clear viscous yellow liquid, after adjusting to pH 6.5 as above, was poured with rapid stirring into 600 ml of EtOH. After standing overnight the precipitated DNA was partially dried by grinding with several changes of EtOH. It was col-

lected on a Büchner funnel employing suction, washed with EtOH and diethylether and dried in vacuo as above. The yields obtained by this method were usually 50 to 55 g of a DNA containing about 7.5% P (purified high-viscosity DNA usually contains about 8.5% P).

Preparation of a DNA-Hydrolyzing Enzyme System from Salmon Kidney

Some 10 years ago it was found that salmon kidney usually contained appreciable amounts of deoxynucleosides as determined by a microbiological procedure (Tarr *et al.*, 1950), and therefore this organ was considered as a potential source of DNA-degrading enzymes. However, other organs were also examined as follows:

Small portions of frozen salmon visceral organs were blended with 2 volumes of water. The extracts were centrifuged 10 to 15 min at 14,000 *g* and filtered through a layer of glass wool to remove insoluble lipids and other floating debris. DNA (10 mg), $MgSO_4$ (0.02*M*) and 3 ml of tissue extract were incubated for 8 to 12 hr at 37°C, hydrolysis of DNA being followed by determination of orthophosphate at intervals by the Gomori procedure. The results indicated that the DNase activity of the tissues studied was in the following order of decreasing activity: kidney, heart, spleen, milt, pyloric caeca, liver and stomach. In view of these results kidney was selected for the following studies.

Kidney tissues of sockeye salmon have been found to contain rather high concentrations of DNA and RNA (Creelman and Tomlinson, 1959). Since the latter, if degraded, would probably yield ribonucleosides which would interfere with isolation of deoxynucleosides, the removal of RNA was obviously necessary with any procedure used for preparing a DNA-hydrolyzing enzyme system from kidney. Several methods for preparing such an enzyme were investigated.

Kidney tissue was blended with 2 volumes of water and the resulting mixture centrifuged 15 min at 12,000 *g* to remove insoluble material. When such extracts were autolyzed for 4 to 12 hr at 37°C, and then dialyzed for 16 to 24 hr against a solution (pH 7.0) containing both cysteine and $MgCl_2$ in 0.001*M* concentration to remove dialyzable degradation products of RNA, they had almost entirely lost their ability to hydrolyze DNA. Adjustment of such extracts to 0.9 saturation with ammonium sulphate at pH 7.0, collection of the precipitate either by centrifugation or overnight filtration through Whatman No. 31 paper, and dialysis as above yielded solutions which were devoid of DNase activity. Even dialysis of the kidney extracts without other treatment resulted in preparations which possessed only a fraction of their original activity.

In other experiments the aqueous kidney extracts were cautiously adjusted to pH 4.5 or 5.0 with 0.2*M* acetic acid and promptly centrifuged. The precipitates were immediately dissolved in 0.05*M* *tris*-(hydroxymethylaminomethane)HCl (*tris*-HCl) buffer pH 8.0. The solutions were practically free from RNA, as determined by the orcinol reaction, but possessed only feeble DNase activity. The neutralized supernatant solutions from the above precipitates also possessed only slight activity. These results indicated that at least one of the enzymes

responsible for degradation of DNA to yield deoxynucleosides and orthophosphate must be very sensitive to the above comparatively mild enzyme fractionation procedures. For this reason the following method, which appeared to cause no loss in specific activity, but which removed RNA successfully, was employed.

Salmon kidney (from spring or sockeye salmon in different experiments) was blended for about 1 min with 3 volumes of H_2O and the suspension centrifuged at 9500 *g* for 15 to 20 min. The supernate (pH about 6.5) was cautiously adjusted to pH 7.0 with 1*N* KOH and 1 volume of 2% protamine sulphate solution (adjusted to pH 5.5 with 1*N* H_2SO_4) was added for each 8 volumes of extract. The precipitate was removed by centrifuging and the clear supernate used immediately since it lost activity when stored at 0°C, also after being frozen (–30°C) and thawed.

The specific activity of these preparations was determined as follows: DNA (30 mg), 0.2 ml 1*M* *tris*-HCl buffer pH 7.5, $MgSO_4$ (0.02*M*) and 9 ml of enzyme preparation were incubated for 6 hr at 37°C, orthophosphate being determined at intervals by the Gomori method as an indication of DNA hydrolysis. The results obtained with two different preparations are given in Table I. Both

TABLE I. Preparation and spec fic activity of a DNA-hydrolyzing enzyme system from salmon kidney. A and B are different preparations.

Fraction	Volume		Protein N		Total units[a]		Specific activity	
	A	B	A	B	A	B	A	B
	ml		*mg*				*units per mg protein N*	
Crude extract	160	285	68	153	32.6	61.8	0.48	0.403
Centrifuge supernate	150	272	51	130	26.6	57.8	0.52	0.445
Protamine treated	132	250	30.4	58	12.7	27.0	0.415	0.465

[a]Amount of enzyme preparation forming 1 μmole of orthophosphate per hour at 37°C.

preparations had closely similar specific activities which were not increased by the fractionation procedure. Determination of ribose by the orcinol reaction showed that about 95% was removed by the protamine treatment. So far, no attempt has been made to separate the different enzymes probably involved in DNA degradation by these crude kidney enzyme preparations.

SALMON LIVER NUCLEOSIDE PHOSPHORYLASE

Previously a nucleoside phosphorylase which exhibited activity toward both purine nucleosides and deoxynucleosides but not toward the corresponding pyrimidine compounds was isolated from lingcod (*Ophiodon elongatus*) muscle (Tarr, 1958). Such enzymes carry out the general reaction:

$$\text{Nucleoside (deoxynucleoside)} + \text{orthophosphate} \rightleftarrows \text{R1-P(DR1-P)} + \text{purine (pyrimidine)}$$

and have therefore been used to prepare the natural (α) forms of the two pentose phosphate esters. In the case of R1-P, preparation of both biologically inactive (β) and active (α) forms has been achieved chemically (Wright and Khorana,

1956; Tener *et al.*, 1957). With DR1-P, chemical synthesis has as yet only resulted in mixtures containing different proportions of the α (active) and β (inactive) forms (MacDonald and Fletcher, 1960). The equilibrium in the above reaction is normally between 15 and 20% in favour of pentose 1-phosphate formation.

In connection with preparation of DR1-P in the present investigation the desirability of obtaining a pyrimidine deoxynucleoside phosphorylase was evident, since DNA degradation would yield both purine and pyrimidine deoxynucleosides, and the lingcod muscle enzyme exhibits no pyrimidine nucleoside phosphorylase activity. Accordingly, the principal visceral organs of salmon were examined to determine whether they exhibited pyrimidine deoxynucleoside phosphorylase activity.

Portions of the various organs were blended with 2 volumes of water, centrifuged, and the supernates tested as follows: Deoxyribonucleoside (0.1 ml containing 100 μg of thymidine or deoxyuridine), 0.02 ml of 1*M* phosphate buffer pH 7.6 and 0.2 ml of tissue extract were incubated for 3 hr at 37°C. Ethanol (9 volumes) was added and the suspension centrifuged 5 min at 10,000 *g*. The supernates were evaporated to dryness over H_2SO_4 and solid KOH. The dry residues were mixed with 0.02 ml water and applied as a number of successive additions with intermediate drying to single starting zones on Whatman No. 1 paper. The papers were developed by descending chromatography for 16 hr at 20°C with *n*-propanol– 28% NH_4OH–H_2O (6:3:1 v/v) (Hanes and Isherwood, 1949) and dried at room temperature. The papers were sprayed with aniline hydrogen phthalate reagent (Partridge, 1949) containing 0.5 ml of concentrated HCl per 100 ml and heated 5 min at 105°C. The presence of both DR5-P and DR1-P was verified by appearance of bright yellow zones which fluoresced strongly in ultraviolet light. These esters moved about 6 and 8 cm respectively under the chromatograph conditions and were not well separated when applied to the paper in crude solution. DR5-P presumably arises as a result of phosphodeoxyribomutase activity. The results of these preliminary tests indicated that the salmon liver preparations were most active with both substrates. Some activity was observed with kidney and spleen extracts, but none with heart, stomach or pyloric caecae.

Since liver appeared to afford the best source of a pyrimidine nucleoside phosphorylase, a purification procedure was sought. Initially, centrifuged aqueous extracts of blended spring or sockeye salmon livers (1 part of liver to 3 of H_2O) were assayed for thymidine phosphorylase activity according to the modified procedure of Friedkin and Roberts (1954a) in which liberation of thymine from thymidine is measured by the optical density of clarified alkaline solutions at 300 mμ. When four different concentrations of extract (0.02 to 0.08 ml) were incubated at pH values of 6.0 and 7.0 for 2 hr at 37°C in a final volume of 1.1 ml using this assay procedure, it was found that the specific activity was between 1.6 and 1.7 (calculated as μmoles of thymine formed per hour per milligram of protein nitrogen).

Attempts to purify the liver nucleoside phosphorylase by ammonium sulphate fractionation of aqueus extracts at pH 7.0, followed by dialysis against 0.001*M* cysteine and $MgCl_2$ (at pH 6.5), or by fractionation at pH 4.5 or 5.0 using dilute acetic acid, yielded preparations which were either inactive or had specific activities considerably lower than those of the crude extracts. Dialysis of freshly prepared extracts against H_2O, 0.1*M* NaCl, 0.1*M* sodium acetate (pH 6.7), 0.1*M* *tris*-HCl buffer pH 7.0, 0.05*M* KCl in 0.01*M* veronal buffer pH 7.4 or 0.05*M* KCl containing 0.001*M* $MgCl_2$ at 0°C for 3 hr caused from 40 to 50% loss of activity as judged by the above assay procedure. After a 16-hr dialysis the preparations had lost all measurable activity while a non-dialyzed control was still fully active.

In view of these results a simple rapid procedure was developed which yielded a heterogeneous preparation adequate for some preliminary studies on the enzyme, and for preparation of DR1-P.

Thawed livers of spring or sockeye salmon which had been obtained from fish shortly after death, and which had been stored several months at −30°C, were used. The liver was cut into small pieces and blended at about 0°C with 2 volumes of water and KF sufficient to bring the final concentration to 0.2*M*. In a typical experiment 100 g of liver was blended with water (200 ml) and KF (5.65 g) at high speed in a Serval Omni-Mixer for 1 min. The suspension was centrifuged at 40,000 *g* for 15 min, and the supernate (pH 6.2) poured through a thick pad of fine glass wool to remove floating particles (chiefly lipid material) (yield 220 ml). The solution was mixed with 37 ml of 2% protamine sulphate solution (pH 5.5) and centrifuged 15 min at 25,000 *g* to yield 252 ml of clear red supernate (pH 6.0). Such preparations may be frozen and stored for at least a month at −30°C without any serious loss in activity.

Initial attempts to determine the specificity of this crude liver enzyme by measuring liberation of orthophosphate from DR1-P in presence of various pyrimidine or purine bases were not very successful. The large amount of protein in the crude preparations rendered the determination of orthophosphate in the presence of very acid labile DR1-P by the method of Friedkin (1950) rather unsatisfactory. For this reason present experiments were confined to a study of formation of DR1-P from known deoxynucleosides and nucleosides using paper chromatographic separation.

Deoxynucleoside (20 μmoles), 1*M* potassium phosphate buffer pH 7.0 (0.02 ml) and enzyme (2.0 ml) were incubated for 2 hr at 37°C in small glass test tubes. The pH of the reaction mixture was 7.0. After adding 0.08 ml of 0.1*N* KOH to bring the pH to about 8.0, the tubes were immersed in a boiling water bath for 3 min with stirring, promptly chilled, and the protein coagulum was removed by filtering through a small conical Whatman No. 1 paper, the coagulated protein being washed with four successive 0.5-ml portions of water. The clear filtrates were evaporated to dryness in 5-ml beakers in an evacuated desiccator over concentrated H_2SO_4 and solid KOH. The residues were dissolved in 0.1 ml of water, and the resulting solution, followed by the washings obtained by rinsing the beakers with a further 0.1 ml of water, streaked along a 12-cm starting line on a 15-cm wide Whatman No. 3 mm paper, using several successive applications with

intermediate drying. The papers were subjected to descending chromatography for 20 hr at 20°C using *n*-propanol–28% NH_4OH–H_2O (6:3:1 v/v). After drying at room temperature a 2-cm wide strip was cut lengthwise from each paper (this represented approximately 16.7% of the material applied). After an examination with ultraviolet light to ensure that deoxynucleosides or nucleosides would not interfere with determination of pentose phosphates, the strips were sprayed first with aniline hydrogen phthalate (Partridge, 1949) and, after drying, heated with phosphomolybdic acid reagent (Hanes and Isherwood, 1949). In this chromatographic system, when purified preparations are used, DR1-P, DR5-P, R5-P and orthophosphate are all separated, the rapidity of their migration being approximately in the above order. R1-P moves at about the same rate as DR5-P. The rates of flow are all reduced when the esters are applied in a rather crude mixture as in the above experiment, and separations are also poorer. Thus there was no clear-cut separation of DR1-P and DR5-P or of R1-P and R5-P, though it was possible to distinguish the individual compounds in the mixtures by application of both the aniline hydrogen phthalate and phosphomolybdic acid sprays.

The zones corresponding to DR1-P plus DR5-P (about 7 cm from origin), and also those corresponding to the ribose phosphates, were cut out as a 3-cm-wide strip and eluted into 10 ml of water for 1 to 2 hr. The eluates were assumed to represent 83.3% of the expected recovery and allowance was made for this in the calculations. Determinations of deoxyribose and ribose were used as indices of the amount of DR1-P and R1-P formed. The results (Table II) showed that

TABLE II. DR1-P and R1-P formation by salmon liver nucleoside phosphorylase.

Substrate	μmoles of:	Amount formed[4]	Results of visual examination of sprayed paper strips
	Deoxyribose phosphates[5]		
Deoxyuridine	5.00	25	++++
	4.62	23	++++
Thymidine	2.8	14	+++
Deoxycytidine	0.77	3.9	++
Deoxyguanosine	0.79	4.0	++
Deoxyinosine	0.66	3.3	++
Deoxyadenosine	0.58	2.9	+
	Ribose phosphates		
Uridine	0.47	2.35	+++
Cytidine	0.25	1.25	++
Guanosine	0.042	0.21	–
Inosine	0.054	0.27	–
Adenosine	0.068	0.34	+
Xanthosine	0.04	0.20	–

[4]Percentage of the 20 μmoles of each substrate used.
[5]The figures given represent the sum of deoxyribose (or ribose) 1- and 5-phosphate esters and are averages of duplicate determinations after subtracting appropriate controls obtained by running experiments without added nucleosides.

the enzyme preparations exhibited deoxyriboside and riboside phosphorylase activity, and that this activity was roughly ten times greater with deoxyribosides under the experimental conditions. The pyrimidine compounds (with the exception of deoxycytidine) were much better substrates than the purine compounds.

These results indicate the very wide specificity of the enzyme preparation. However, since the preparation has not yet been purified, it is possible that more than one nucleoside phosphorylase system may be involved. In this connection it is of interest that horse liver deoxynucleoside phosphorylase is apparently a single enzyme (Friedkin, 1950; Friedkin and Roberts, 1954a and b), which synthesizes deoxynucleosides from thymine, uracil, hypoxanthine and guanine in presence of DR1-P.

Phosphopentosemutase Activity of the Nucleoside Phosphorylase

The above experiments showed that the crude salmon liver nucleoside phosphorylase preparation was able to form pentose 5-phosphates from the corresponding pentose 1-phosphate esters, and therefore contained both phosphoribomutase and phosphodeoxyribomutase activities. The rate of formation of DR5-P from DR1-P was determined as follows:

A reaction mixture containing 100 μmoles of thymidine, 0.1 ml of 1*M* potassium phosphate buffer (pH 7.0) and 9.9 ml of crude nucleoside phosphorylase enzyme was incubated at 37°C, 1.0-ml samples being removed at intervals and heated immediately for 3 min in a boiling water bath. The samples were then treated as in the previous experiment. The zones on the chromatograms which corresponded to DR5-P and DR1-P (about 6 and 8 cm from origin) were eluted together into 10 ml of water. After 2 hours standing with occasional mixing, deoxyribose, labile phosphorus (Gomori method) and "total phosphorus" (after heating samples for 45 minutes at 100°C in 0.1*N* H_2SO_4 in order to hydrolyze the more acid stable DR5-P) were determined, using appropriate duplicate aliquants. The results (Table III) showed that there was comparatively rapid

Table III. Rate of formation of DR1-P and DR5-P from thymidine.

Time	Deoxyribose	Total P	Labile P	Ratio of deoxyribose to total P[6]	DR1-P[7]	DR5-P[7]
minutes	μmoles	μmoles	μmoles			
0	*	*	*	–	–	–
15	1.40	1.25	0.89	1:0.89	71	29
30	1.35	1.24	0.66	1:0.92	53	47
60	1.37	1.25	0.62	1:0.91	49	51
120	1.34	1.58	0.51	1:1.15	37	63
180	1.05	1.16	0.34	1:1.10	29	71
240	1.10	0.97	0.11	1:0.88	11	89
480	1.12	1.20	0.11	1:1.08	9	91

[6]Theoretical ratio is 1:1.
[7]Calculated from the difference between acid labile and total phosphorus.
* = Not appreciable.

formation of DR1-P since 1.4 μmoles of deoxyribose phosphates (i.e. 14% of the 10 μmoles of thymidine used) were formed within 15 min. Thereafter there was a slow increase in the proportion of DR5-P, the final equilibrium being about 90% in favour of this more acid-stable ester. This value is similar to that reported for phosphoribomutase in the literature, and that recently found for lingcod muscle phosphoribomutase (Martin and Tarr, 1961).

Preparation of Deoxynucleosides

The method used was based on that described by Anderson *et al.* (1952). Initial experiments showed that since DNA hydrolysis was comparatively slow, it was necessary to retard bacterial growth. Under the conditions used (pH about 7.5 at 37°C) chlortetracycline (10 μg/ml) proved unsatisfactory due to its instability and toluene was therefore employed as a bacteriostat.

In one experiment 11 g of DNA (6.6% P) was dissolved in 200 ml of hot water and added, together with 49 g $Mg_2SO_4.7H_2O$ (0.02*M* final concentration) to 3.8 l of crude protamine-treated salmon kidney enzyme preparation, the pH of which had been cautiously adjusted to 7.6 with 1*N* KOH. The initial orthophosphate content (121 μg/ml) rose to 226 μg/ml during a 30-hr incubation at 37°C under a layer of toluene. This indicated formation of 420 mg of orthophosphate, equivalent to 58% hydrolysis of the DNA. In a second similar experiment 15 g of DNA (7.58% P) was hydrolyzed under the above conditions with 4.2 l of kidney enzyme in a total volume of 4.4 l for 45 hr at 37°C. The orthophosphate concentration increased from 136 to 268 μg/ml, equivalent to formation of 580 mg of orthophosphate or 51% hydrolysis of the DNA.

The two preparations were pooled, filtered by gravity through Whatman No. 31 paper, and evaporated, using a rotary evaporator (37°C), to a viscous brown liquid (about 60 ml). Ethanol (340 ml) was added with stirring and the slurry (85% EtOH content) shaken overnight at 25°C. The suspension was centrifuged at 9500 *g* and the supernate set aside. The residue was mixed with sufficient water to bring the volume to about 60 ml, and again extracted with EtOH (340 ml) for 4 hr by shaking. The centrifuging and extraction were repeated once more, and the alcohol extracts were pooled.

The combined extracts were evaporated under reduced pressure as above to a thick syrup. The syrup was diluted with water to 150 ml and mixed with 75 g of acid-washed Norit A charcoal (Pfanstiel Labs. Inc., Waukegan, Ill.). The deoxynucleosides (which are quantitatively adsorbed) were eluted from the charcoal on a 15-cm-diameter Büchner funnel with 8 l of 50% EtOH containing 1% of 28% NH_4OH employing suction. This procedure removed all but two free amino acids (presumably aromatic compounds) which occurred in the digest and were adsorbed by the charcoal. These two amino acids appeared later in the eluate from the Dowex 2×8 formate column (see below).

The charcoal eluate was evaporated as above, the pH adjusted to 10.5 with 28% NH_4OH solution, and the solution (340 ml) filtered through Whatman No. 1 paper. The clear yellow solution was run by gravity on to a 200–400-mesh

Dowex 2×8 formate resin column 32 cm long and 2.8 cm diameter, and the column was washed with 500 ml of water. It was eluted under slight pressure at 250 ml/hr employing a gradient in which 12 l of 0.4*M* ammonium formate (pH 8.6) was run into 12 l of water which was adjusted to pH 9 with NH_4OH. Twenty-millilitre fractions were collected and checked for absorption at 260 mμ (after appropriate dilution) and for their deoxyribose content (0.1-ml portions using the cysteine–H_2SO_4 method). The results are shown in Fig. 1.

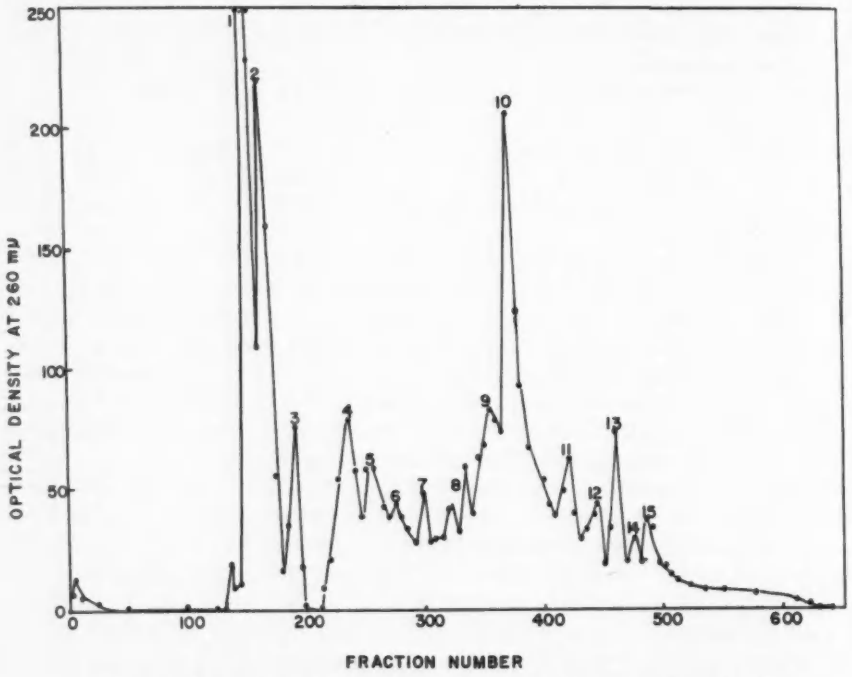

FIG. 1. Separation of deoxynucleosides on a Dowex 2 × 8 formate column.

In Fig. 1, Peak 1 (O.D. at 260 mμ was 290) contained thymidine and Peak 2 deoxyuridine. Peak 3 was an amino acid (unidentified). Peak 4 contained both guanine and hypoxanthine deoxyribosides, and Peak 5 hypoxanthine deoxyriboside. All fractions between 139 and 175 and between 215 and 280 contained deoxyribose, the remainder being deoxyribose-free. The fractions indicated under Peaks 7 to 15 inclusive were pooled so that nine solutions were obtained. These will be referred to later.

The fractions noted above which comprised Peaks 1 to 6 were pooled as indicated in Table IV and the five solutions thus obtained were evaporated to small volumes which were set aside at 0°C to permit crystallization of the deoxynucleosides or amino acids. Data concerning the isolation of these compounds are

given in Table IV. All absorption data are given in mμ. Thymidine was recrystallized once from anhydrous methanol. Deoxyuridine, which was contaminated with an amino acid, was purified as follows. The pooled fractions, after removing most of the water using a rotary evaporator, were dried in high vacuum over P_2O_5. The residue was ground with 10 ml of anhydrous methanol

TABLE IV. Preparation of deoxynucleosides.

Fraction No.[8]	Crystallization volume	Yield of product	O.D. ratios[9] in 0.01*N* HCl 250/260	280/260	Purity: calc.[10] from ε max. in 0.01*N* HCl	Compound
	ml	*mg*			%	
1	10	1,100	0.65 (0.65)	0.75 (0.72)	90 (267)	Thymidine
(145–160)	recrystallized from 10 ml methanol	900	0.62 (0.65)	0.71 (0.72)	101 (267)	"
	filtrate evaporated to 5 ml yielded a crystalline residue at 0°C.	475	–	–	–	Amino acid unidentified
2						
(161–180)		1,770	0.76 (0.72)	0.38 (0.37)	60 (262)	Deoxyuridine
	recrystallized	505	0.73 (0.72)	0.35 (0.37)	97.5 (262)	"
	O.D. ratios in 0.01*N* NaOH		0.825 (0.81)	0.34 (0.31)		
3						
(181–200)	150	200	–	–	–	Amino acid unidentified
4						
(201–245)	150	240	1.63 (1.63)	0.19 (0.23)	83 (249)	Hypoxanthine deoxyriboside
	75 (filtrate)	350	0.98 (1.02)	0.71 (0.70)	65 (255)	Guanine deoxyriboside
	Contaminating ammonium formate was removed under high vacuum at 45°C.					
		215	0.98 (1.02)	0.71 (0.70)	95 (255)	Guanine deoxyriboside
5+6	50	620	–	–	57 (249)	Hypoxanthine deoxyriboside
(246–290)	Contaminating ammonium formate removed under high vacuum at 45°C.					
	50	300	1.62 (1.63)	0.18 (0.23)	90 (249)	"

[8]Fractions which were pooled are given in parentheses.
[9]The values in parentheses are those given by the California Corporation for Biochemical Research, Los Angeles, in a chart on "Properties of the nucleic acid derivatives".
[10]The wave length used in calculating the ε max. values is given in parentheses.

and the crystalline residue (amino acid) removed by filtration. The filtrate was evaporated under reduced pressure to a gum (1.77 g) (Table IV). The gum was dissolved in 5 ml of warm methanol, applied to eight 46-cm sheets of washed Whatman 3-mm paper in long streaks and developed (descending 16 hr at 20°C) successively as follows with intermediate drying. (A) 1% of 28% ammonium hydroxide in water-saturated *n*-butanol, 16 hr. (B) *n*-propanol, 28% ammonium

hydroxide, water (6:3:1 v/v). The deoxyuridine which was well separated from a contaminating amino acid and traces of other ultraviolet-absorbing compounds was eluted from the paper strips with 90% EtOH. The eluates were dried under reduced pressure (rotary evaporator) and the deoxyuridine crystallized, after drying over P_2O_5, from 2 ml of anhydrous methanol at 0°C. All four deoxynucleosides were isolated in comparatively pure state and were identified by their characteristic ultraviolet absorption spectra, their O.D. ratios at 250/260 and 260/280, their absorption maxima, and finally by their chromatographic properties in three solvent systems (Table VI).

The fractions representing Peaks 7 to 15 inclusive (Fig. 1) were pooled, evaporated under reduced pressure to small volumes, and the ammonium formate was removed under high vacuum at 45°C (Anderson *et al.*, 1952). Fraction 7 yielded 40 mg of hypoxanthine (about 85% purity) O.D. ratios 250/200 = 1.47; 260/280 = 0.059 (0.04) and fraction 8 yielded 100 mg of hypoxanthine of similar purity as judged spectroscopically.

The pooled fractions composing Peaks 9 to 15, which were first assumed to be deoxynucleotides from their chromatographic behaviour, were not identified. When small amounts (1 to 10 O.D. units at 260 mμ) were subjected to electrophoresis in 0.05*M* sodium citrate buffer pH 3.5, using Whatman No. 3 paper (30 v/cm), these seven peaks each yielded two to five separate ultraviolet-absorbing substances which migrated anodically from 2 to 14 cm in 2 hr. The distances travelled are similar to those exhibited by nucleoside mono-, di- and triphosphates toward the cathode under the same conditions. On chromatography in *iso*-propanol–28% NH_4OH–H_2O (7:1:2 v/v) (Razzell and Khorana, 1959), the peaks yielded from two to five distinct ultraviolet-absorbing zones (Rf values between 0.3 and 0.6). A number of these electrophoretically or chromatographically separated substances were eluted from the paper with 0.01*N* HCl and their absorption in the ultraviolet region was determined as usual. While absorption was quite high in the range 230 to 280, none of the substances gave O.D. ratios 250/260 and 280/260 which were at all typical of known purines, pyrimidines or their nucleoside or nucleotide derivatives. Thus the following ratios (250/260 and 280/260 respectively) were recorded for major ultraviolet-absorbing zones from electrophorograms: Peak 9, 0.85 and 0.75; Peak 10, 0.75 and 0.79; Peak 12, 0.88 and 0.77; and Peak 13, 1.29 and 0.52. The eluates of several typical zones both from electrophorograms and paper chromatograms gave negative tests for deoxyribose (Stumpf, 1947) and none contained measurable phosphorus after H_2SO_4 hydrolysis (Umbreit *et al.*, 1949). No further attempts were made to identify these substances.

Preparation of Purines and Pyrimidines

It was noticed that when digests similar to the above were incubated in absence of toluene, bacterial action commenced after about 8 to 10 hr and was severe after 16 hr. In digests so prepared, deoxyribose, as determined by the Dische reaction, was usually either absent or present in comparatively small amounts, indicating that hydrolytic (or phosphorolytic) cleavage of the deoxy-

nucleosides had occurred. Paper chromatography showed that such extracts contained free purines and pyrimidines, and could therefore be used as a source of these bases. Two such digests were prepared and used for isolation of purines and pyrimidines as follows, the general conditions being those employed in preparing the deoxynucleosides except that no toluene was used.

In one experiment, 17 g DNA (6.1% P) was dissolved in 200 ml of hot water and incubated for 5 hr at 37°C with 5 l of kidney enzyme and $MgSO_4$ in 0.02*M* concentration. The mixture was then allowed to stand for 16 hr at 22°C. In another experiment 14 g DNA (7.5% P) was incubated with 4.6 l of enzyme for 12 hr at 37°C followed by gradual cooling for 8 hr at 0°C. Analyses indicated that 90% of the DNA-P had been liberated as orthophosphate in the first experiment, and 87% in the second. Both extracts emitted unpleasant odours indicative of incipient bacterial putrefaction. The solutions were evaporated, extracted with 85% EtOH, charcoal treated and subjected to Dowex 2×8 formate chromatography under the same conditions as those employed in the isolation of the deoxynucleosides. The results are given in Fig. 2 and Table V. Thymine, uracil,

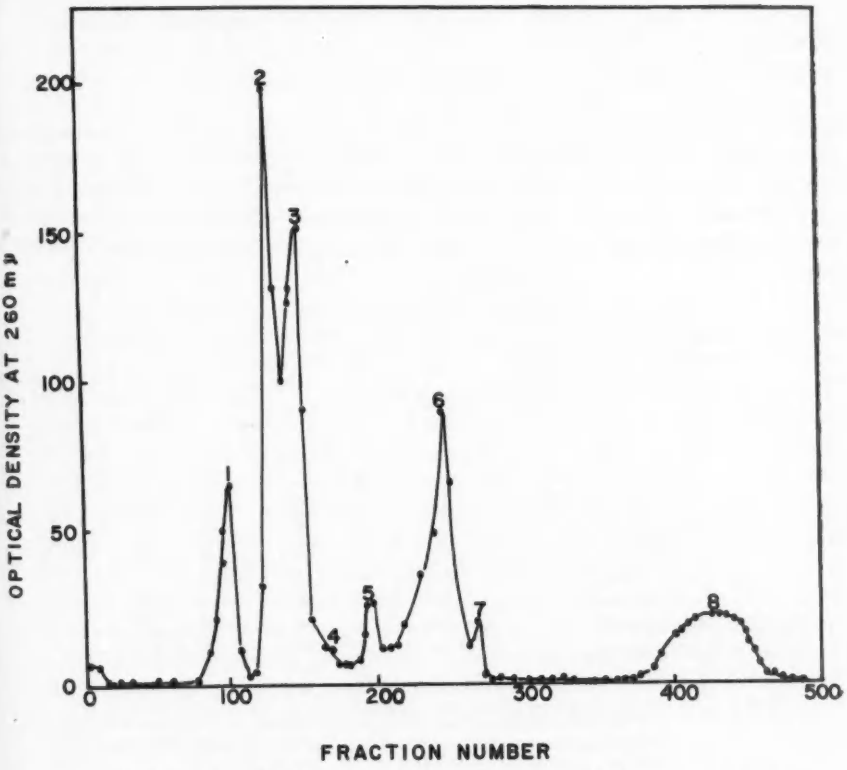

FIG. 2. Separation of purines and pyrimidines on a Dowex 2 × 8 formate column.

TABLE V. Preparation of purines and pyrimidines.

Fraction No.[8]	Crystallization volume (ml)	Mg of product	O.D. ratios[9] in 0.01*N* HCl 250/260	O.D. ratios[9] in 0.01*N* HCl 280/260	% Purity; calc.[10] from ϵ max. in 0.01*N* HCl	Compound
1 (75–110)	10	40	–	–	–	Amino acid (unidentified)
2	50	375	0.69 (0.67)	0.50 (0.53)	95 (264)	Thymine
(115–140)	20	75	0.70 (0.67)	0.48 (0.53)	95 (264)	Thymine
3	80	160	0.88 (0.84)	0.17 (0.17)	94 (259)	Uracil
(141–145)	25	80	0.85 (0.84)	0.17 (0.17)	89 (259)	Uracil
4 (156–175)	35	110	–	–	–	Amino acid (unidentified)
5 (176–205)	10	180	1.61 (1.63)	0.20 (0.23)	93 (259)	Hypoxanthine deoxyriboside
6 (206–247)	45	370	1.44 (1.45)	0.057 (0.04)	94 (248)	Hypoxanthine
7 (248–270)	20	145	1.47 (1.45)	0.52 (0.04)	90 (248)	Hypoxanthine
8 (370–480)	90	115	0.53 (0.56)	0.59 (0.6)	93 (267)	Xanthine

Footnotes as in Table IV.

hypoxanthine and xanthine (presumably arising from guanine deamination) were isolated in a reasonably pure state as judged by their ultraviolet absorption spectra, their paper chromatographic propeties in three solvent systems (Table VI) and their "ϵ max." values. A small amount of hypoxanthine deoxyriboside was also obtained and, as with the deoxynucleoside preparations, two unidentified amino acids.

TABLE VI. Rf values of isolated deoxynucleosides, purines and pyrimidines compared with reference compounds (about 50 μg of each chromatographed). Rf values at 20°C in three solvent systems[11] using Whatman No. 1 paper. A = compound isolated. B = reference compound.

	1		2		3	
	A	B	A	B	A	B
Thymine	.68	.68	.74	.72	.715	.715
Thymidine	.78	.77	.87	.89	.63	.63
Uracil	–	–	.82	.80	.54	.54
Deoxyuridine	.74	.74	.83	.83	.51	.51
Deoxyguanosine	.52	.55	.79	.80	.54	.54
Hypoxanthine	–	–	.62	.62	.56	.57
Deoxyinosine	.70	.70	.82	.825	.53	.53
Xanthine	–	–	.62	.62	–	–

[11]1 = 2-hour ascending development in 5 ml *iso*-propanol + 5 g $(NH_4)_2SO_4$ + 100 ml water (Friedkin, 1955). 2 = 2-hour ascending development in ammonia water pH 10.0 (Wyatt, 1955). 3 = 16-hour descending development in *iso*-butyric acid, 75; water, 25; and 28% ammonia hydroxide, 1.3 (v/v).

PREPARATION OF DEOXYRIBOSE 1-PHOSPHATE

In previous work (Tarr, 1958) DR1-P was prepared in good yield using deoxyguanosine as substrate. The liver nucleoside phosphorylase described above exhibited greatest activity with the pyrimidine deoxynucleosides of uracil and thymine as substrates, and therefore an investigation of the formation of DR1-P with this enzyme was undertaken. Initial studies in which reaction mixtures containing 10 μmoles of thymidine and orthophosphate per millilitre of crude salmon liver nucleoside phosphorylase (3.9 mg of protein N per ml) showed that the enzyme had a specific activity corresponding to about 0.025 μmole of DR1-P formed per minute per milligram of protein N at 37°C. One major difficulty with this enzyme was occasioned by the presence of the phosphodeoxyribomutase activity, which has so far not been eliminated. DR1-P and DR5-P are difficult to separate by ion-exchange chromatography unless borate complexing is employed. Consequently, in the present work conditions were employed which would tend to minimize DR5-P formation, and, since this compound does not form a crystallizable dicyclohexylammonium salt, advantage was taken of this fact in isolation of DR1-P.

Two millimoles (484.5 mg) of thymidine prepared from salmon DNA, 50 ml of crude salmon liver nucleoside phosphorylase and 1.5 ml of 1*M* potassium phosphate buffer pH 7.0 were incubated for 40 min at 37°C. After adding 0.5 ml of 28% NH_4OH the mixture was heated to 90°C in a boiling water bath, chilled to 0°C, the protein removed by filtration and the residue washed on the funnel with 100 ml of water. The combined filtrates were evaporated to 15 ml using a rotary evaporator (37°C) and passed through a 200–400-mesh Dowex 50×8 NH_4^+ ion-exchange resin column 10 cm long and 1.9 cm diameter. The column was washed with 100 ml H_2O, the eluate and washings were evaporated to approximately 2 ml under reduced pressure (pH > 7.0) and 6 ml of 28% NH_4OH and 12 ml of *n*-propanol were added. After shaking mechanically for 1 hr the suspension was filtered through Whatman No. 4 paper on to the top of a 27- by 3.4-cm cellulose powder column. This column was prepared by suspending 75 g of Whatman ashless cellulose powder in a solution containing *n*-propanol–28% NH_4OH–H_2O (6:3:1 v/v) and packing this in layers, as usual. The column was washed with 500 ml of the solvent before applying the above solution, and was eluted with the same solvent at a flow rate of 1.4 ml/min, collecting 12.5-ml fractions. The fractions were assayed for deoxyribose (Stumpf method) and labile P (Gomori method) using 0.1-ml portions from which the solvent was removed in vacuo before making the determinations. Fractions 15 to 25 inclusive which gave strongly positive tests for deoxyribose but not for labile phosphorus were pooled and evaporated to dryness. The residue was dissolved by warming in 10 ml of anhydrous methanol, filtered and set aside at 0°C. The crystalline mass was collected on a tared sintered glass filter (yield 260 mg). The O.D. ratios were: 250/260, 0.65; and 250/280, 0.71; which are characteristic of thymidine. The purity as calculated by the "ϵ max." when dissolved in 0.01*N* HCl was 97%.

Fractions 27 to 31 inclusive gave positive tests for both deoxyribose and labile phosphorus. There was some indication of slight separation of deoxyribose 1- and 5-phosphates but this was not sufficient to permit clear-cut separation. The fractions were pooled, and, after adding 1 millimole of barium acetate, were evaporated to dryness under reduced pressure. The dry product was taken up in 5 ml of water. The insoluble residue was removed by centrifuging, washed with water, the solutions were pooled and 30 ml of EtOH was added, care being taken to keep the pH above 7.0. The precipitate was collected by centrifuging, suspended in 80% EtOH, centrifuged and dried 3 hr over P_2O_5 in high vacuum. The yield was 122 mg of crude mixed barium salts of deoxyribose 1- and 5-phosphates. Analysis for deoxyribose, labile and total phosphorus indicated that the material contained only 43% of the theoretical amounts (calculated on the basis of MW = 350) and that about 23% occurred as DR5-P and 77% as DR1-P. When a small portion of the crude material was examined by paper chromatography after removing the barium by means of Dowex 50×8 NH_4^+ resin, both DR1-P and DR5-P were identified.

The crude barium salt was dissolved in 5 ml of H_2O and filtered through Whatman No. 4 paper on to a 200–400-mesh Dowex 50×8 cyclohexylamine ion-exchange column 5 cm long and 1.9 cm diameter, the filter and column being washed with 25 ml of water. The column eluate was evaporated to dryness using a rotary evaporator (30°C). The residue was dissolved in 1.0 ml water-saturated *n*-butanol and diethyl ether (4 ml) was added gradually at 0°C. Crystallization did not occur. The pale yellow precipitate was dried in high vacuum over P_2O_5 after the solvent was decanted. The yield was 36 mg. Analysis showed that the material contained 74% of the expected amount of deoxyribose, 73% of the total phosphorus and 71% of labile phosphorus expected for dicyclohexylammonium DR1-P (MW 412). Only a single zone corresponding to DR1-P was found by paper chromatography, DR5-P being absent.

In a second preparation 1.15 g of crude mixed deoxynucleoside syrup (alcohol extraction step from a DNA digest) was incubated for 1 hr at 37°C with 100 ml of liver enzyme and excess orthophosphate. In this case the ion-exchange resin isolation procedure employed in previous work (Tarr, 1958) was used. Only 90 mg of crude mixed barium deoxypentose phosphate salts of about 47% purity as calculated by the deoxyribose and total phosphorus content was obtained. This material contained about 30% of DR5-P and 70% DR1-P as determined by the ratio of labile to total phosphorus, and gave two distinct zones characteristic of these esters on paper chromatography. The product was not further purified.

DISCUSSION

Recent reviews have summarized present knowledge regarding enzymic hydrolysis of DNA (Schmidt, 1955; Laskowski, 1959). The lability of the salmon liver crude enzyme preparation used in the present studies has complicated purification. Thus the possible participation of a number of enzymes such as a

DNA depolymerizing endonculease, of phosphodiesterase enzymes yielding either 3′- or 5′-deoxymononucleotides (Tener *et al.*, 1959) and of phosphomonoesterases remains to be determined. Where bacterial contamination was largely avoided by use of toluene the important end products of action of the crude kidney enzyme on DNA were deoxynucleosides. The nature of the ultraviolet-absorbing fractions which were eluted subsequent to the purines, pyrimidines and deoxynucleosides was not determined. Deoxynucleotides were not found in these fractions.

Salmon liver nucleoside phosphorylase was found to activate a wide range of nucleosides. However, its activity was greater toward the pyrimidine compounds deoxyuridine and thymidine than toward the other substrates studied. The enzyme showed the following order of activity toward other deoxynucleosides investigated: deoxyguanosine > deoxycytidine > deoxyinosine > deoxyadenosine > uridine > cytidine > adenosine > inosine > xanthosine. In this connection it is interesting to note that Friedkin (1950) found that horse liver thymidine phosphorylase exhibited activity toward both inosine and adenosine, as well as toward thymidine.

Salmon liver nucleoside phosphorylase exhibited an instability similar to that found with the kidney DNase enzyme preparation. However, the enzyme worked actively with no sign of hydrolytic activity toward the nucleoside substrates (i.e. no detectable free deoxyribose or ribose formation) in presence of 0.2*M* KF. Perhaps KF could be used as a stabilizing agent during purification. This enzyme preparation also possessed both phosphoribomutase and phosphodeoxyribomutase activity which complicated attempts to isolate DR1-P from reaction mixtures. Attempts to prepare this ester in pure crystalline form were unsuccessful, though it was isolated as an amorphous product about 74% pure. The original preparation method of Friedkin, which gave good preparations with the horse liver enzyme, was not studied. In any case, much better yields of both crystalline deoxyribose 1-phosphate and ribose 1-phosphate dicyclohexylamine salts can be obtained with lingcod muscle nucleoside phosphorylase (Tarr, 1958).

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A Comparison of Three Methods of Inactivating Lobster Claws[1]

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ABSTRACT

Mortalities during 4½ months among 4 lots of 50 lobsters, 3 with claws inactivated, were: plugged 54%, banded 56%, cut tendons 78%, untreated 98%. No appreciable loss of claws, bands or plugs occurred. Plugged lobsters are more susceptible than banded to infection with with *Gaffkya homari*, the agent causing lobster blood disease. The advantages of banding outweight the disadvantages.

INTRODUCTION

To reduce injuries and mortalities among live lobsters during storage and shipment, the crusher claw or both claws are inactivated. In Europe this is accomplished by fastening twine, brass wire or rubber bands around the claws (Thomas, 1958). In North America the most common method is to insert a small wooden or plastic plug in the thumb joint. In a few areas some fishermen cut the extensor tendons so the lobsters are unable to open their claws. Rubber bands are also used to a limited extent.

Each of these methods has certain advantages and disadvantages. Plugging is effective, cheap and fast. In time, however, the tissue near the plug turns dark and with prolonged storage the shell may erode quite extensively. Baird (1950) showed that the wounds caused by plugging were almost invariably infected with a gram-negative rod bacterium. He also showed that injections of this bacterium were lethal to lobsters. This suggests that plugging may cause some mortality in storage. It also seemed possible that the wounds may be sites of infection for *Gaffkya homari*, a gram-positive micrococcus, the causative agent of lobster blood disease (Snieszko and Taylor, 1947). European lobster dealers contend that plugged lobsters lose their claws more frequently than banded lobsters. Banding, however, is a relatively slow operation and this is possibly the Canadian fishermen's chief objection. In addition, Canadian dealers contend that bands slip off during storage. Templeman (1948) felt that cutting the extensor tendons showed promise but considered that further study was required.

To obtain further information on the effects of plugging, banding and tendon-cutting during long-term storage two experiments were conducted.

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MATERIALS AND METHODS

For the *first experiment*, 211 unplugged, live, 2-clawed lobsters were obtained on March 24, 1958, by special arrangement direct from the fishermen at Port Maitland, Yarmouth County, Nova Scotia. They averaged 1.2 lb (0.54 kg) in weight and ranged in carapace length from $3\frac{1}{8}$ to $4\frac{1}{2}$ inches (80 to 114 mm). The lobsters were shipped the next day in dry wood shavings to St. Andrews, New Brunswick. During shipment both claws of each lobster were banded with light rubber bands. On arrival on March 25, the lobsters were found to be in excellent condition with only one weak and one dead.

On March 27, the 200 lobsters that remained in excellent condition were divided into 4 lots of 50. One lot was plugged, another banded, and the tendons of the third were cut. The fourth lot was untreated and served as a control. Each lobster was measured and tagged with a serially numbered, metal and rubber carapace tag (Wilder, 1954) before treatment. Each lot was placed in a square tank 3 × 3 ft (92 × 92 cm), filled to a depth of 12 inches (30 cm), and supplied with enough running sea water and compressed air to maintain the dissolved oxygen near the saturation level. This degree of crowding, about 1 lb per imperial gallon (1 kg per 10 litres), is well within the limits of commercial practice. At the time of treatment, 3 of the lobsters in each lot were one-clawed.

The plugs were machine-made of basswood, $1\frac{1}{2}$ inches long, $\frac{1}{4}$ inch wide (38 × 6.4 mm), and $\frac{3}{16}$ inch (4.8 mm) thick at one end, tapering to a point at the other. The upper surface was smoothly rounded, the lower surface flat. Both claws of each lobster in a lot of 50 were plugged by inserting a plug under the shell of the claw in such a manner that the blunt end of the plug protruded at the thumb joint and so inactivated the claw.

The rubber bands were 1 inch long, $\frac{1}{2}$ inch wide and about 1/25th of an inch thick (25 × 13 × 1 mm). These were difficult to stretch with the fingers of one hand and it was most convenient for one person to hold the lobsters, while another applied the bands. The bands were placed around the widest part of the claw near the base of the thumb (Fig. 1).

The extensor tendons of both claws in a lot of 50 were cut carefully with the point of a sharp scalpel as described by Templeman (1948).

The four tanks were checked daily, the temperature recorded and any dead lobsters removed. These were examined and the extent of their injuries recorded. Loss of bands or plugs was also noted. At approximately weekly intervals all lobsters were removed from each tank and examined individually. At about 3-week intervals the lobsters were fed salted herring. This experiment ran for $4\frac{1}{2}$ months, ending August 8, 1958.

The *second experiment* was conducted to determine whether plugged lobsters were more susceptible to infection with *Gaffkya homari* than banded lobsters. On August 18, 1960, during an epidemic of blood disease in southwestern Nova Scotia and in Charlotte County, New Brunswick, 140 recently-caught, unplugged

FIG. 1. Condition of rubber bands when applied (left) and after use for 8 months (right).

lobsters were obtained from the central Northumberland Strait area. They averaged 0.73 lb (0.33 kg) in weight and ranged in carapace length from $2\frac{1}{2}$ to $3\frac{3}{16}$ inches (64 to 81 mm). At the time, there was no evidence of a blood disease epidemic in Northumberland Strait and no unusual commercial mortalities have since been reported there. Blood smears were, however, prepared on August 22 from 59 other lobsters received in the same shipment from the same area. When examined, 40 were vigorous, 10 weak and 9 dead. One vigorous, 2 weak and 1 dead were very lightly infected with *Gaffkya*. The low incidence and lightness of the infection suggest that the lobsters became infected after they reached St. Andrews. The 40 vigorous lobsters were stored in a separate tank of running sea water throughout the experiment.

On August 18, both claws of half the lobsters were plugged with hand-made white pine plugs, $1\frac{3}{8}$ inches long and $\frac{1}{4}$ inch wide (35 × 6.4 mm). Both claws of the remainder were banded with narrow rubber bands, $\frac{7}{8}$ inch long, $\frac{1}{8}$ inch wide and $^1/_{25}$ inch thick (22 × 3 × 1 mm), considered strong enough to inactivate the claws of these small lobsters. The 140 lobsters were placed in a cylindrical fibreglass tank, 6 ft inside diameter and 3 ft high (183 × 91 cm) filled to a depth of $2\frac{1}{2}$ ft (76 cm) with running sea water. The experiment proper was started on

August 22 when 2 dead lobsters heavily infected with *Gaffkya homari* were placed in the tank for the lobsters to feed on. At this stage 69 plugged and 67 banded lobsters were alive and vigorous. Additional dead infected lobsters were suspended in the tank in a mesh bag on August 23, 25 and 31. Apart from the 2 dead lobsters placed in the tank on August 22, the lobsters were not fed during the course of the experiment, which was continued until October 11. The tank was checked daily, the temperature recorded and the dead lobsters removed. Several of these dead lobsters were so badly mutilated they were discarded without further study. Blood smears prepared from the remainder were stained with Gram's stain and examined under oil immersion for the presence of *Gaffkya*.

RESULTS

First Experiment

The weekly mortalities among the plugged, banded, tendon-cut and untreated lobsters are listed in Table I. The percentage accumulative mortalities are plotted in Fig. 2. Total mortalities over the 4½-month period were as follows: plugged 54%, banded 56%, cut tendons 78% and untreated 98%. Moulting was not a factor in these deaths.

Table I. Weekly mortalities in lots of 50 lobsters with both claws plugged, banded, tendons cut and untreated.

Week ending	Av. water temp.	Plugged	Banded	Tendons cut	Untreated
	°C				
April 3	4.2	0	0	2	4
10	4.2	1	2	3	2
17	4.9	2	0	1	2
24	7.1	2	2	2	2
May 1	7.2	3	2	1	1
8	6.8	3	2	3	5
15	7.3	2	2	1	8
22	8.2	0	1	2	5
29	9.1	0	2	0	0
June 5	9.5	0	0	2	0
12	10.2	1	0	0	6
19	9.8	0	1	1	1
26	10.2	2	0	3	7
July 3	11.7	0	1	3	1
10	12.6	0	1	3	1
17	12.8	1	2	5	1
24	13.6	2	4	5	1
31	13.4	5	5	1	2
Aug. 7	14.0	3	1	1	0
Totals		27	28	39	49

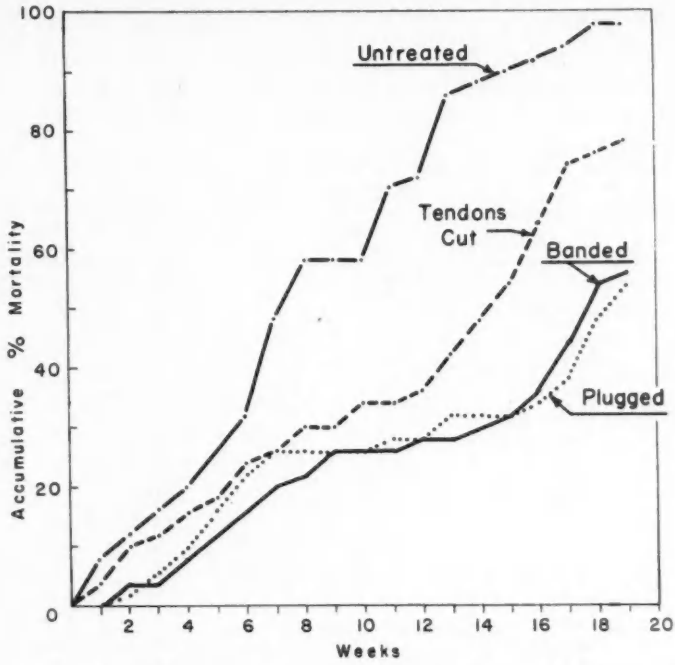

FIG. 2. Accumulative weekly percentage mortalities in lots of 50 lobsters with both claws plugged, banded, tendons cut and untreated.

The tendon-cut lobsters bled quite freely in air for almost a minute. The loss of blood, measured for 7 lobsters averaged 15 cc. No bleeding was evident among the plugged lobsters.

The untreated lobsters lost 12 claws during the course of the experiment, the plugged lobsters 2 claws, the banded and tendon-cut lobsters none.

Only one of the ½-inch wide rubber bands came off and only 2 plugs came out during the 4½-month period.

After the close of the experiment proper, the survivors were held alive but given less detailed attention. From August 8 to 13, on September 30 and on November 7th, 8, 4 and 2 of the plugged lobsters respectively were boiled. Most showed a blackening of the shell, meat and extensor tendon in the vicinity of the plug. Several showed quite severe erosion of the shell near the point of insertion of the plug. It was noted that the blackened meat of the two examined on November 7 was definitely off-flavour.

By December 2, 1958, after 8 months' storage, both the rubber bands (Fig. 1) and the wooden plugs had deteriorated appreciably.

Second Experiment

The daily mortalities among the plugged and banded lobsters exposed to *Gaffkya homari*, the numbers examined and the numbers found to be infected are listed in Table II. Percentage accumulative mortalities to October 11 are

Table II. Numbers of plugged and banded lobsters dead and infected each day after exposure to *Gaffkya homari*.

Date	Temperature	69 Plugged			67 Banded		
		Dead	Examined	Infected	Dead	Examined	Infected
	°C	*no.*	*no.*	*no.*	*no.*	*no.*	*no.*
Aug. 22	14.5	0	0	0	0	0	0
23	14.4	0	0	0	0	0	0
24	14.1	3	3	3	0	0	0
25	14.1	14	14	14	2	2	2
26	14.0	10	10	9	2	2	2
27	14.4	4	1	1	1	1	1
28	14.8	6	6	6	4	3	3
29	14.5	4	2	2	2	1	1
30	14.6	3	1	1	2	2	1
31	14.5	5	4	4	3	3	3
Sept. 1	14.8	1	1	1	6	3	3
2	14.3	3	2	1	2	2	1
3	14.0	2	2	2	1	1	0
4	14.0	1	1	1	4	3	3
5	13.8	4	4	4	2	2	2
6	13.3	2	1	1	4	4	4
7	13.8	1	1	1	2	2	2
8	13.5	0	0	0	2	2	2
9	14.0	0	0	0	2	2	2
11	14.0	0	0	0	1	1	1
12	14.0	0	0	0	2	2	2
13	14.5	0	0	0	1	1	0
16	14.9	0	0	0	2	2	2
18	14.6	1	0	0	0	0	0
19	13.8	0	0	0	1	1	1
22	14.0	0	0	0	1	1	1
Oct. 3	13.1	0	0	0	2	2	2
7	12.5	0	0	0	1	1	1
Totals to Oct. 11		64	53	51	52	46	42

plotted in Fig. 3. A photomicrograph of a typical blood smear from an infected lobster is shown in Fig. 4. It is obvious that the plugged lobsters died faster and suffered higher mortalities (93%) than the banded (78%). Moulting was not a serious factor in these deaths as only one banded lobster moulted during the experiment. Appreciably lower mortalities occurred among the 40 vigorous lobsters from the same shipment that were not exposed to *Gaffkya* during storage. Only 14 (35%) of these lobsters were dead by October 11, a mortality rate similar

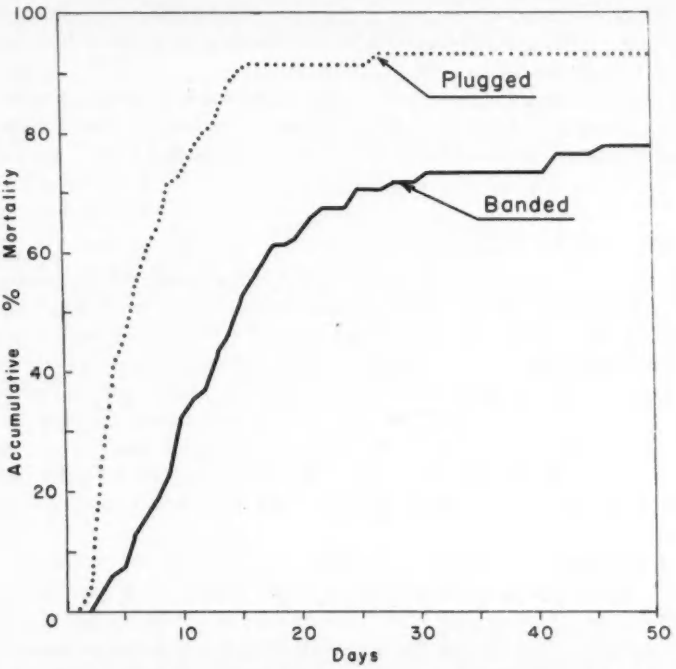

FIG. 3. Accumulative daily percentage mortalities among plugged (69) and banded (67) lobsters when exposed to *Gaffkya homari*.

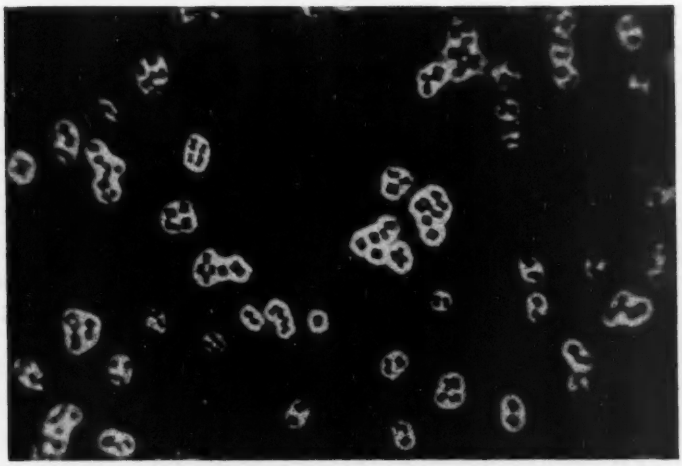

FIG. 4. Typical blood smear from lobsters infected with *Gaffkya homari*. Magnification 890×. Photomicrograph by P. W. G. McMullon.

to that which occurred in the first experiment conducted at considerably lower temperatures. Of the dead plugged lobsters examined for *Gaffkya*, 96% were found to be infected; of the banded, 91%.

The narrow bands used on the smaller lobsters in this experiment were not strong enough to inactivate all the claws completely. During the experiment these bands were lost and replaced quite frequently.

DISCUSSION AND CONCLUSIONS

It is clear that to reduce mortalities among impounded lobsters some method of inactivating the claws is necessary. Where blood disease is not a factor, banding and plugging are equally effective in reducing mortalities and both methods are superior to tendon cutting. The tendon-cut lobsters did not appear to be adversely affected by the operation and no sudden, heavy mortalities occurred. It seems unlikely, therefore, that the loss of blood which accompanied the tendon cutting contributed appreciably to the higher mortality in this group. At times, particularly during handling, the claws of these lobsters opened when they came in contact with others. Injuries inflicted at such times undoubtedly caused some mortality.

No appreciable loss of claws resulted from any of the three treatments. Apparently this is not a serious problem if the lobsters are handled carefully. Loss of plugs or suitable bands is not serious over a 4½-month period if they are properly applied. The narrow bands used in the second experiment are not suitable for commercial use.

A serious disadvantage of the wooden or plastic plugs is the damage they do to the claws. Not only is the appearance of the shell and meat adversely affected but some meat is destroyed and off flavours develop. Since mortalities among the plugged and banded lobsters in the first experiment did not differ appreciably, there is no reason to believe that a rod bacterium associated with plugging (Baird, 1950) caused appreciable losses in this experiment. There is, however, good evidence that plugged lobsters are more susceptible than banded ones to infection with *Gaffkya homari*, the causative organism of lobster blood disease. For these reasons, the use of plugs to inactive lobster claws is not recommended.

The advantages of banding the claws with rubber bands seem to outweigh the disadvantages. Although bands are now slow to apply, it seems probable that simple efficient techniques for applying them can be developed. One new technique has recently been described by Taylor (1960).

ACKNOWLEDGMENTS

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Dietary Marine Fish Oils and Cholesterol Metabolism 3. The Comparative Hypocholesterolemic Activities of Fish Oil and Vitamin A[1]

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ABSTRACT

Certain fish liver oils, when present in the diet, prevented hypercholesterolemia in chicks produced by cholesterol feeding. The hypocholesterolemic activity of the oils was proportional to the amount incorporated into the diet. Vitamin A-enriched corn oil produced similar results but corn oil itself was without effect. It was concluded that vitamin A was responsible for 73 to 85% of the activity of the fish liver oil. The cause of the additional activity of the marine oils is at present unknown.

INTRODUCTION

Previous work in this laboratory indicated that the hypocholesterolemia induced in chicks by cholesterol feeding was prevented by dietary fish oils such as lingcod liver oil and halibut liver oil (Wood and Biely, 1960a). The hypocholesterolemic factor was located in the unsaponifiable portion of the oil (Wood and Biely, 1960b) and its indentity tentatively suggested as Vitamin A (Wood, 1960). However, the evidence for this postulation was unsatisfactory in some respects. For instance, when crystalline vitamin A acetate was incorporated into the cholesterol-containing diet in place of fish liver oil, its hypocholesterolemic activity was not so great as might be expected. Moreover, the correlation between activity and amount of dietary vitamin A was rather indefinite. The present studies were therefore undertaken to elucidate whether vitamin A was indeed the active agent in the fish liver oils.

MATERIALS AND METHODS

Birds

Day-old white leghorn cockerels were placed for one week on the control diet. At this point birds whose body weights deviated widely from the average were discarded and the remainder distributed in groups of sixteen per cage. The groups were then placed on the appropriate test diets for 14 days.

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Diets

The control diet was formulated as shown in Table I. Additions to this diet listed in the accompanying Tables and Figures were substituted isocalorically for the sucrose. The diets were stored at 2°C and sufficient food for one day

Table I. Composition of the control diet. The caloric value of the diet = 3.6 calories/g.

	%		%
Ground wheat	41.20	Distillers' solubles	1.87
Cornmeal	7.50	Dehydrated grass	0.94
Ground oats	3.75	Iodized salt (fine)	0.37
Middlings	3.75	Ground limestone	0.75
Bran	3.75	Manganese sulphate	0.01
Soybean meal	5.62	2250A, 300D, vitaminoil	0.20
Fishmeal	1.50	Nicarbazin	0.04
Meatmeal	3.75	Sucrose	25.00
Riboflavin 50 mg/100 lb feed			

only was placed in the feed troughs. The amount supplied to the chicks was adjusted so that the calorie intake was the same on all diets. The average food intake during the fourteen-day test period varied from 13 to 15 g/chick/day depending on the diet.

Determination of Serum Cholesterol

The birds were starved for 18 hours prior to the collection of the blood from the brachial vein. Samples from four birds were pooled and the serum collected in the normal manner. The total cholesterol concentration in the serum was determined using the method of Sperry and Webb (1950).

RESULTS

The experimental cockerels were distributed into six groups. One group was fed the control diet, a second group the control diet + 1% cholesterol, and the remaining four groups the control diet + 1% cholesterol + respectively 1, 2, 3 and 4% lingcod liver oil with a potency of 42,500 I.U. vitamin A/g. The serum cholesterol levels of the chicks on the various diets are shown in Fig. 1. The broken line indicates the cholesterol concentration in chicks fed the control diet. The presence of 1% cholesterol in the diet increased the mean serum cholesterol concentration from 194 mg/100 ml to 686 mg/100 ml. This increase was prevented by the dietary fish liver oil, the hypocholesterolemic effect being proportional to the amount of oil incorporated into the diet. The presence of 4% oil prevented almost completely the increase in serum cholesterol.

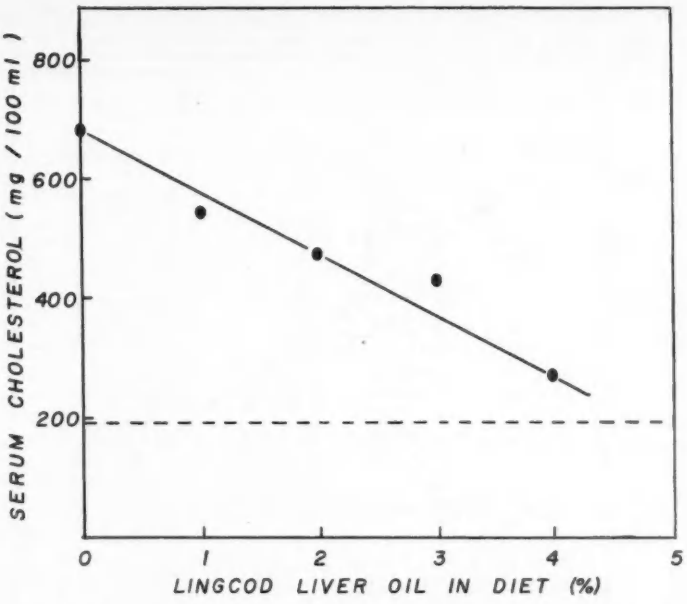

FIG. 1. The prevention of hypercholesterolemia in cholesterol-fed chicks by different amounts of dietary lingcod liver oil.

A similar experiment was carried out using a vitamin A-enriched corn oil instead of the lingcod liver oil. The enriched oil was prepared by dissolving crystalline vitamin A acetate in corn oil until the potency was 42,500 I.U. vitamin A/g oil. The results obtained with the enriched corn oil (Fig. 2) were similar to those obtained with the fish oil. The hypocholesterolemic properties of the diets increased with the concentration of the oil so that a 4% level in the diet was sufficient to afford almost complete protection against the effect of the dietary cholesterol.

Two different samples of lingcod liver oil varying greatly in their vitamin A content were tested for hypocholesterolemic activity. The oils were incorporated into cholesterol-containing diets in different amounts but the total quantity of vitamin A added was the same in both cases. The effects of the diets on serum cholesterol levels are shown in Table II. The hypocholesterolemic action of the fish oil-containing diets appeared to depend on the vitamin A content rather than on the amount of oil in the diet. The incorporation of corn oil into the diets at the 4 and 9% levels was without effect on serum cholesterol concentration.

The above experiments, although demonstrating the similar effects of fish oils and vitamin A-enriched corn oil, did not yield a direct comparison of the oils because the experiments were carried out independently. To clarify this

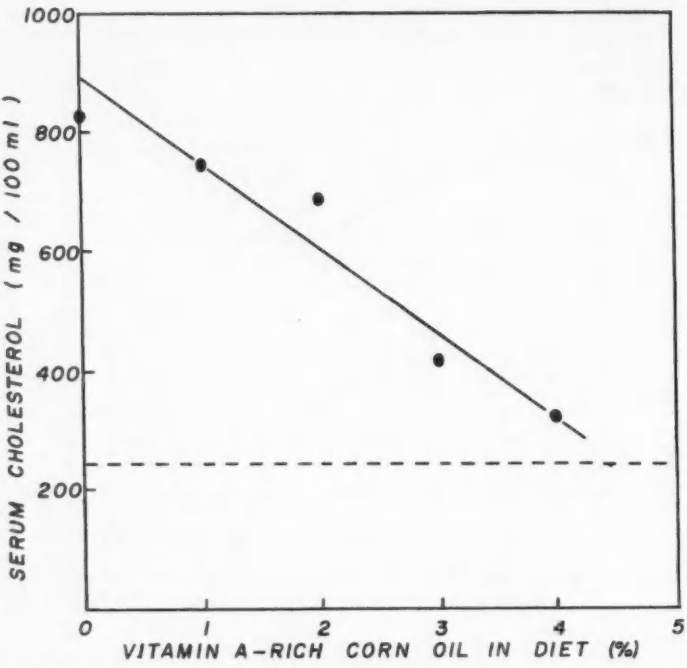

FIG. 2. The prevention of hypercholesterolemia in cholesterol-fed chicks by different amounts of dietary vitamin A-enriched corn oil.

TABLE II. Comparative effect of the same total amount of vitamin A from two different lingcod liver oils on serum cholesterol levels in cholesterol-fed chicks.

Addition to cholesterol-containing diet[2]	Vitamin A potency (I.U./g oil)	Mean serum cholesterol (mg/100 ml)
None	–	990 ± 68
4% corn oil	–	980 ± 88
9% corn oil	–	948 ± 146
4% lingcod liver oil	42,500	176 ± 34
9% lingcod liver oil	18,900	179 ± 23

[2]Cholesterol-containing diet was the control diet + 1% cholesterol.

point, vitamin A-enriched corn oil was compared directly with various fish oils (Table III). In every instance the enriched corn oil was less effective in preventing hypercholesterolemia than was the fish liver oil with a comparable vitamin A content. Statistical analysis showed that the difference was significant

TABLE III. Comparative effect of fish liver oils and vitamin A-enriched corn oil on serum cholesterol levels in cholesterol-fed chicks.

Expt. no.	Number of birds per diet	Addition to cholesterol-containing diet[2]	Vitamin A potency (I.U./g oil)	Mean serum cholesterol (mg/100 ml)	Relative calorie intake	Relative weight gain	Relative hypocholesterolemic activity[3]
1	16	10% dogfish liver oil	8,300	435 ± 86	100	100	138
	16	10% corn oil	8,300	618 ± 156	105	131	100
2	64	10% dogfish liver oil	7,300	306 ± 95	100	100	–
	64	10% corn oil	7,300	483 ± 133	98	136	–
3	16	4% lingcod liver oil	42,500	214 ± 33	100	100	119
	16	4% corn oil	42,500	312 ± 133	106	120	100
4	16	9% lingcod liver oil	18,900	179 ± 23	100	100	118
	16	9% corn oil	18,900	301 ± 114	103	114	100

[2]See footnote to Table II.

[3]Hypocholesterolemic activity is designated; mean serum cholesterol level for birds on cholesterol-containing diet minus mean serum cholesterol level for birds on the same diet plus the oil. The relative activities were calculated taking the enriched corn oil diet as 100.

at the 1% level. Although the calorie intakes of the chicks on the two diets were similar, the weight gains of the chicks were always greater on the enriched corn oil diets. Moreover, there appeared to be an inverse relation between the weight gain of the chicks and the hypocholesterolemic activity of the oil supplements.

DISCUSSION

The results presented here show that the hypocholesterolemic effect of fish liver oils can be duplicated by vitamin A-enriched corn oil, although corn oil itself is without effect. It has also been shown that the amount of vitamin A incorporated into the diet is more important from the view of preventing hypercholesterolemia than is the amount of fish oil itself. Moreover, since corn oil has no effect on serum cholesterol levels, the observed hypocholesterolemic effects of different amounts of vitamin A-enriched corn oil must be due to the vitamin A content. In other words, the degree of prevention of hypercholesterolemia is proportional to the vitamin A content of the diet. The rather indefinite relation observed previously (Wood, 1960) was due probably to the fact that at that time the vitamin A was added in the dry state and a less homogenous diet with respect to the vitamin was therefore obtained. Although the results discussed above indicate that vitamin A is the major hypocholesterolemic agent in fish liver oils, only 73 to 85% of the activity of the oils can be attributed to their vitamin A content (calculated from the relative hypocholesterolemic activities in Table III). The reason for the additional activity is at present unknown.

It is interesting to note the inverse proportionality between the hypocholesterolemic action of the diets and the weight gain of the chicks. Since the calorie intake was similar on the different diets, the fish oil may therefore exert its "extra" effect by lowering the absorption of food including the cholesterol. On the other hand, the additional activity may be due to another hypocholesterolemic agent in the fish oil, to the presence in the oil of a substance which increases the action of vitamin A, or perhaps to a form of vitamin A in the fish oil which is more active than the acetate form used to fortify the corn oil. Further investigations are being carried out to determine the reason for the additional activity of the fish oils.

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Radioactive Iron as a Fish Mark[1]

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ABSTRACT

Experiments designed to test the feasibility of using radioactive iron as a fish mark are described. The biological half-life (turnover) of iron in speckled trout (*Salvelinus fontinalis*) was found to be probably at least 2 years. Iron-59, with a physical half-life of 45.1 days, was used in the experiments.

INTRODUCTION

Much investigation of the contamination of fish with radioisotopes has been done, in particular, by Japanese workers. The source of this contamination has been fallout from nuclear explosions mainly, but some has come from effluents from nuclear reactors. The use of radioisotopes as fish marks has, however, received little investigation, probably because of difficulties in obtaining the necessary materials. Two recent Russian investigations should be mentioned. Bogoiavlenskaia (1959) investigated the use of calcium-45 as a mark for fish. The mark obtained appeared to have a useful life of approximately one year, using their counting technique. It should be noted that Ca-45 concentrated almost entirely in the bony structures of the fish, such as gill covers, scales and axial skeleton. Calcium-45 emits a single relatively soft beta ray with no associated gamma emission. Reading the live fish, therefore, could be extremely difficult as the sample count approached the same level as background. The physical half-life of Ca-45 is 164 days; this is sufficiently long for most experiments. Karzinkin, Soldatova and Shekhanova (1959) marked 106,000 young sturgeon with phosphorus-32 by feeding them oligochaetes (*Enchytraeus albidus*) which had been fed on either a flour suspension or hydrolyzed yeast containing the radioisotope. These authors found that the mark remained useful for 2.5-3.0 months, using their counting technique. Phosphorus-32 has only a 14.3 day half-life and would not normally be useful for a long-term experiment.

Of the 775 radioisotopes of elements 1–92 inclusive, listed by Bradford (1957), very few can satisfy these criteria. All alpha ray emittors must be

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eliminated because of the biological potency of these particles; the majority of the remainder are eliminated because of too short a physical half-life or biological incompatability, such as chemical toxicity, energetic radiation and organ concentration.

Of the potentially useful radioisotopes, radioactive iron appears to be the most likely to be useful. Two radioisotopes exist; iron-55 has a physical half-life of 2.94 years and decays by electron capture, the only emission being the characteristic X-ray of the daughter manganese-55. Iron-59 has a physical half-life of 45.1 days and decays by negatron emission with emission of three different gamma rays from the daughter cobalt-59 nucleus (Overman and Clark, 1960, pp. 7–10). Iron-59 was chosen for the work to be described, since the background level in our laboratory is quite high; distinction between background count and the gamma rays of Fe-59 is immensely simpler than separating the X-ray associated with decay of Fe-55. However, since the radioisotopes are chemically identical, results obtained are applicable to both.

MATERIALS AND METHODS

High purity Fe-59 was obtained for the experiments from the Oak Ridge National Laboratory, Oak Ridge, Tenn. Table I shows the specifications of the material.

TABLE 1. Specifications of Fe-59 solution obtained from Oak Ridge National Laboratory. Data given are for the original 1-millicurie (mc) shipment; subsequent shipments varied somewhat in specific activity, concentration, and Fe-55 and Co-60 content.

Chemical form:	$FeCl_3$ in HCl solution
Acidity:	1.98 N
Concentration:	3.98 ± 5% mc/ml
Specific activity	8378 mc per gram of iron
Concentration Fe-55:	0.064 mc/ml
Concentration Co-60:	—0.0018 mc/ml
Total Fe per mc:	0.1425 mg

On receipt, the solution was diluted to a concentration of 5 microcuries per millilitre (μc/ml) with a highly acid (pH 2.25) solution of acriflavine hydrochloride. The acriflavine was found to be necessary to prevent infection in the fish when the solution was injected into the body cavity. Ferric chloride is hydrolyzed to the hydroxide in all but highly acid solutions; ferric hydroxide is not soluble in water of normal pH range, and thus the highly acid solution was necessary to maintain the radioisotope in solution. The fish apparently did not suffer

from the effects of the injection. The injections were made through the ventral body wall just anteriad of the ventral fins. Great care was taken to avoid puncturing any of the organs. It was found that 10-cm trout could be injected with the solution at the rate of 5 per minute; accuracy was achieved by the use of 1-ml tuberculin syringes with No. 25 needles. No anaesthetic was necessary.

Two methods of radio-assay of the specimens were used. In Method 1, the tip of a Nuclear-Chicago Corporation DS8-1 6-mm needle scintillation probe was placed over the heart-liver region of the fish's belly and the counts made with a portable ratemeter. In Method 2, the fish were kept in an annular tank with the detector (a special gamma-ray-sensitive Geiger-Muller tube) at the centre so that the fish were always in the same geometrical relationship to the probe. Counts were registered by a laboratory ratemeter, and recorded on a strip-chart recorder continuously. Provided that a radioisotope emitting hard gamma rays is being used, the second method is much superior to the first, since the fish are not handled. However, where a yes or no answer is required, or where error due to variations in geometry between fish and detector can be tolerated, the first method enables many more fish to be handled. It is also the field method of choice. Figures 1 and 2 show the apparatus for radio-assays by each method.

FIG. 1. Radio-assay of Group I trout, using needle scintillation probe.

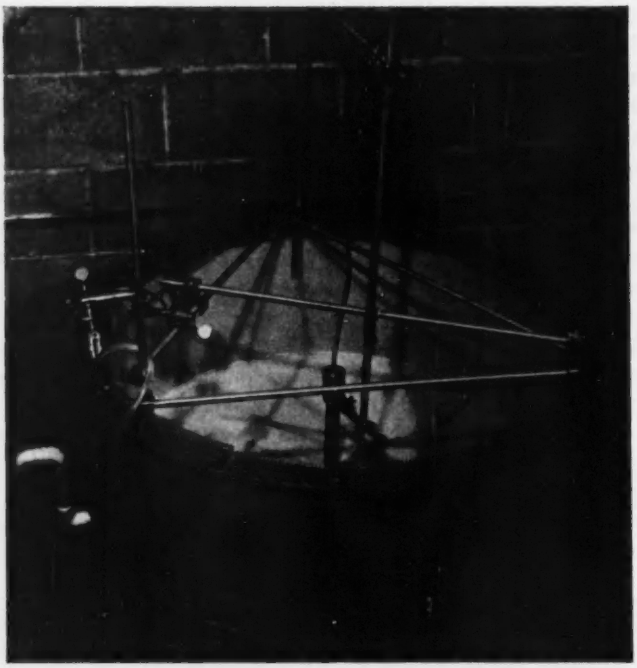

FIG. 2. Constant-geometry tank for radio-assay of Group II trout.

The loss of an element in a biological system, that is, turnover, follows the exponential decay law. Thus, the effective half-life of a radioisotope in a fish is the combined product of the physical decay and the biological loss. The relationship between effective half-life (T_e), physical half-life (T_P), and biological half-life (T_B) is:

$$\frac{1}{T_e} = \frac{1}{T_P} + \frac{1}{T_B} \tag{1}$$

The effective half-life of the isotope in the experimental fish was obtained by calculating the slope (b) and the Y-axis intercept ($\log N_0$) of the least-squares line relating the logarithm of the count rate ($\log N_t$) to time (t), for each fish assayed (cf. Fig. 3, which however shows geometric mean values for each day):

$$\log N_t = \log N_0 - bt$$

Half-life (T_e) was then calculated from the slope using the expression:

$$T_e = \frac{\log 2}{b} = \frac{0.3010}{b} \tag{3}$$

RESULTS

GROUP I

Twenty-five *Salvelinus fontinalis* were injected with 1 ml of Fe-59 solution, containing 1.0 μc of Fe-59, on July 4, 1960. The total ferric chloride concentration was 0.576×10^{-9} g/ml. The trout were kept in a wire cage floating in running water at 10°C. Radio-assays of the individual fish were started on July 5 by Method 1 and continued until October 3, when a failure occurred in the

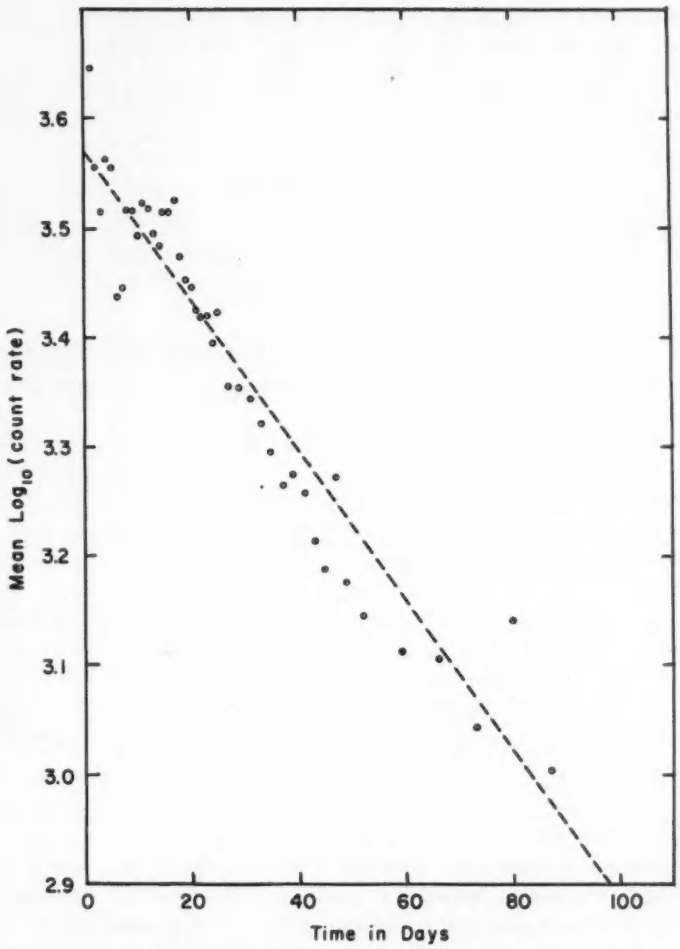

FIG. 3. Relationship between $\log_{10}$(count rate) and time for Group I trout, from radio-assay by the needle scintillation probe over the heart-liver region. Values plotted are geometric means for all fish assayed on each day.

laboratory's dechlorinating system and all specimens died. Eight of the specimens had died from handling and partial chlorine intoxication prior to this date. The relationship between count rate and time (Fig. 3) has a slope of –0.006893 log units per day (Table II), and the corresponding effective half-life is, from (3), 43.7 days. From (1), the biological half-life is estimated as approximately

TABLE II. Analysis of radio-assay data for trout of Group I and Group II.

Statistic	Symbol	Group I	Group II
Number of individual radio-assays	n	823	515
Average time of assay, days	t	25.6	3.447
Average of logarithms of counts per minute	$\log N_t$	3.393	2.6882
Regression coefficient (slope)	b	–0.006893	–0.006984
Standard error of b	. . .	0.000530	0.000391
Correlation coefficient	r	–0.413	–0.714
Probability of significance	P	—0.01	—0.01
Y-axis intercept	$\log N_0$	3.569	2.7123
Effective half-life, days	T_e	43.7	43.1
Physical half-life, days	T_P	45.1	45.1
Biological half-life, days	T_B	1408	978
95% confidence limits of T_B, days	. . .	241, ∞	332, ∞

1408 days. There was no evidence during the experiment that the radioisotope had any effect on the trout; deaths from handling and chlorine in other tanks were as numerous.

Considerable day-to-day variation occurred in the counts of this group. Minor changes in geometrical relationship of detector and source, i.e. error in probe placement, caused much of this, and there was also the effect of the fish's struggles during the readings. There was an apparent engorgement of the heart-liver region as a result of these struggles, since a passive fish showed a lower count than if it were struggling. However, most of these variations averaged out, and had little effect on the slope of the regression line. Also, despite equal amounts of radioisotope injected into each fish, there was much individual variation. Differences in size resulted in differing dilutions of the isotope, and also resulted in different geometrical relationships with the detector. The 5% confidence limits on the estimate of biological half-life are 241 days to infinity.

GROUP II

The effect of handling the trout in Group I daily for the first month and less frequently afterwards might have had some effect on the turnover of iron. In order to check this possibility, a group of 6 trout, approximately 17 cm long, were marked with 10-μc each of Fe-59 and were placed in the annular tank mentioned previously. Continuous recordings of the radioactivity level of these fish were made, and the data taken from the strip chart. The regression analysis

(Table II) yielded an effective half-life of 43.1 days and thus a biological half-life of 978 days. The 5% confidence limits on the latter place the biological half-life between 332 days and infinity. It should be noted that the dosage of 10 μc was apparently too much, in that 2 trout died on the 30th day and the remaining 4 on the 32nd day after injection.

DISCUSSION

In order that a radioisotope may be useful as a fish mark in the field, three criteria must be satisfied. These are:

1. the element must have a slower turnover rate in the fish, i.e., a long biological half-life in relation to the fish's longevity;

2. a radioisotope of the element must exist whose physical half-life is reasonably long in relation to the fish's longevity;

3. the radioisotope must emit radiation which is reasonably detectable with field equipment, but at the same time, with a low enough energy transfer to the fish so that little or no biological damage is done.

By using iron-59, it has been shown that iron has a very long biological half-life in trout, i.e., a slow turnover rate. Iron-59 has a rather short physical half-life of 45.1 days, but another radioisotope of iron, Fe-55, has a half-life of 2.94 years. Iron-55 does have the disadvantage of a very weak emission, the 5.9-kilovolt characteristic X-ray of the daughter Mn-55. Detection of this X-ray entails the use of an X-ray spectrometer; with the advent of transistorized instruments, this requirement presents no particular difficulty. Assuming therefore that Fe-55 is used, a life-time mark for most species of fish should be possible; it is unlikely that other species of fish would show biological half-lives of iron much different from trout.

Aside from possible detection difficulties, two other points should be mentioned. First, in the present study, the radio-iron was introduced into the fish by injection. Although this is probably the most efficient method in terms of equality of mark and maximum economy of radioisotope, it is only useful where the fish are large enough to be handled. Trout as short as 8.0 cm were successfully injected in trials, but the losses from handling and damage from the needle were prohibitive in smaller fish. Introduction of the radio-iron through the food for smaller fish would probably be successful, although possibly wasteful of the isotope. This method would also result in considerable contamination of the tank and waste water system, since it is likely that the fish would not take up all the available iron. The method used in Bogoiavlenskaia's (1959) experiments, where Ca-45 was absorbed directly from the surrounding water, will not work for iron. Iron salts are quickly hydrolized in water of normal pH range, and most of the material precipitates out.

The second point lies in the fact that fish grow, and hence the specific activity of the radio-iron in the blood goes down even though there may be very slow physical and biological loss. For this reason, long-term marks made by introducing radio-iron into very small fish could be rendered extremely difficult to detect if the fish grow very large.

One possible drawback to the use of Fe-55 as a mark lies in the presence of this radioisotope in nuclear fallout. The probability of interference from fallout radio-iron is small at the present time, but should nuclear testing be resumed, it could have serious consequences.

Another possible complication arising from the use of any radioisotope mark lies in the possibility that some of the radioisotope will be transferred to a predator eating the marked fish. Provided that only interspecific predation occurs, this transfer could be advantageous, in determining the amount of predation. However, intraspecific predation could result in a spread of a mark across several year-classes and thus could abscure results.

For short-term marking Fe-59 is much to be preferred. The characteristic gamma rays of this isotope make it extremely easy to identify with portable gamma spectrometers, and these instruments can detect very low levels of radioisotope by practically eliminating background interference. The only limitations associated with Fe-59 are the increased hazard of biological damage to the specimen, and the relatively short physical half-life. The length of time which a mark will persist will depend entirely on the sensitivity of the detecting equipment, the dilution factor of growth, and the original amount of radioisotope in the fish. The mark never vanishes completely, since the decay is exponential both physically and biologically. Thus, in the long run, the limit in time of usefulness is entirely dependent on the intrumentation.

To summarize, iron turnover in trout is quite slow; a mark using radioactive iron is thus possible for fish over 8 cm long by injecting either Fe-55 or Fe-59. In smaller fish some method of introducing the radioisotope through the food must be employed. With Fe-55, a lifetime mark should be achieved, provided extremely sensitive and selective radio-assay equipment is used. Short-term marks, probably up to a year, could be achieved using Fe-59, detection being by means of a gamma spectrometer.

Possible dangers to humans from consumption of radioisotope-marked fish can exist, depending on the radioisotope used and the amount in each fish. Assuming 1.0 μc of Fe-59 in each fish, and also assuming that the whole fish were eaten by a man with 100% absorption of the iron, it would require 20 fish to equal the maximum permissible body burden for Fe-59, as specified in Handbook 69, National Bureau of Standards (1959), for occupational exposure. Using one-tenth this level (2 μc) for the general public, only 2 fish eaten would supply the maximum permissible body burden. However, most of the Fe-59 in the fish is in the blood, and would largely be removed during cleaning; also absorption of iron from food is not perfect and probably rarely exceeds 10%. Thus, there would be little likelihood of danger to man from the consumption of fish marked

with Fe-59, provided marking levels were low, and a low percentage of the population were marked. For Fe-55, the maximum permissible burden for total body is 3 millicuries; since the marking would still be 1 microcurie per fish, danger to man becomes negligible.

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Plasma Proteins of Coho Salmon, *Oncorhynchus kisutch*, as Separated by Zone Electrophoresis[1,2]

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ABSTRACT

Plasma proteins, as separated by zone electrophoresis, of coho salmon have been investigated from an early age to spawning and death. A protein fraction which may be similar to γ-globulin in other vertebrates increased throughout the period studied. Smolt transformation was associated with a temporary disappearance of the fastest migrating protein fraction. A lipoprotein fraction, presumably serum vitellin, was associated with egg formation. Serum vitellin was absent from the plasma of males, immature females, spawning females and spawned-out females.

INTRODUCTION

Egg production in oviparous vertebrates is associated with many changes in the plasma proteins of the female. These protein changes are under hormonal control and can be induced in immature animals of either sex or in castrates by exogenous estrogens. Laskowski (1935) and Roepke and Hughes (1935) demonstrated that sera from laying fowl contained a phosphoprotein, serum vitellin, which was not present in non-laying female or male birds and was absent from mammalian sera. Subsequent investigators have demonstrated the presence of serum vitellin during egg formation or following estrogen administration in pigeons (McDonald and Riddle, 1945), in ducks (Mandel *et al.*, 1947), in reptiles (Laskowski, 1936; Dessauer and Fox, 1959) and in fishes (Laskowski, 1936; Bailey, 1957).

Dr G. S. Ridgway (personal communication, 1959) investigating geographically correlated differences in sockeye salmon found an antigenic serum component in mature or maturing females which was absent from males. This antigenic character was also found in high concentrations in salmon eggs. Similar components have been found in mature female carp sera (Uhlenhuth and Kodama, 1914; quoted by Sasaki, 1932) and have been extensively studied in fowl in which serum vitellin and ovovitellin are serologically related (Roepke and Bushnell, 1936; Hosoda, *et al.*, 1955).

Free and zone electrophoretic analyses of avian serum proteins have shown that egg formation or estrogenization is associated with the appearance of a lipoprotein containing lipid phosphorus but not protein phosphorus (McKinley *et al.*, 1953) and pre-albumin components (Moore, 1948; Brandt *et al.*, 1951; Heim and Schechtman, 1954) in addition to serum vitellin. At the same time there is

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[2]Paper No. 2 concerning the physiology and behaviour of salmonid fishes, from the Fisheries Research Board of Canada Biological Station, Nanaimo, B.C. Paper No. 1 of this series appeared in the *Canadian Journal of Zoology*, **38**: 199–202, 1960.

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a temporary disappearance of α_1-globulin together with its associated lipid (McKinley *et al.*, 1954; Vanstone *et al.*, 1955).

The plasma proteins of non-avian egg laying vertebrates have not been extensively investigated in relation to sex or physiological state by electrophoretic methods. Dessauer and Fox (1959) demonstrated that two slow moving fractions in plasma of viviparous snakes were mainly responsible for increased plasma protein levels during estrus. These fractions may be similar to the PP zone and fraction 8 of Vanstone *et al.*, (1955) or the P_2 and P_1 fractions of McCully *et al.* (1959) which were identified tentatively as lipovitellin and lipovitellenin complexes. Drilhon (1954 a, b) working with carp obtained results suggesting that maturing females contain greater amounts of albumin and α-globulin than males and that lipids are associated with albumin and β-globulin in maturing females.

A preliminary investigation of the plasma proteins of coho salmon (*Oncorhynchus kisutch*) is the subject of the present report and forms a part of the study of the physiology and behaviour of salmon outlined by Brett (1957). No attempt has been made to present quantitative results since a pure race of fish was not examined and it has been found that the relative amounts of certain protein fractions vary between races of coho salmon (unpublished observations). Similar differences in plasma protein pattern have been demonstrated between races of man (Smithies, 1955), of snakes (Dessauer and Fox, 1958) and of sockeye salmon (Mr I. H. Carlson, personal communication, 1960).

MATERIALS AND METHODS

Yearling presmolting coho and yearling coho smolts were reared from eggs under laboratory conditions. In addition some smolts were captured at the beginning of their seaward migration in the Great Central Lake and Sproat Lake drainage system on the west coast of Vancouver Island. Prepuberal 2-year-old fish were obtained by trolling in Georgia Straits off Nanaimo, and maturing fish were captured at the beginning of their spawning migration or on the spawning grounds of the watershed of Great Central and Sproat Lakes.

Blood samples were obtained from the caudal artery and vein by amputating the tail and collected in heparinized bottles. The whole blood was stored on ice and transported to Nanaimo where the plasma was obtained by centrifugation. All samples were in the electrophoretic apparatus within 6 hours of sampling.

The ages of all sea-going and maturing fish in the early part of their migration were determined by scale readings. Due to scale resorption it was found impractical to obtain scales from spawning fish.

Zone electrophoresis employing aqueous veronal buffer (pH 8.6, ionic strength 0.05) was carried out as described by McKinley *et al.* (1954) except insofar as the papers were supported on a sheet of pebbled Plexiglass (Sehon *et al.*, 1956) and were

completely moistened with buffer before the plasma samples were applied. The proteins were fractionated for 18 hours at a current of 0.5 mA/cm and the developed electropherograms were then dried at room temperature. Proteins on strips cut from the electropherograms were stained with Azocarmine B (Harders and Van Mulken, 1953) without prior extraction with fat solvents. Other strips cut from the same electropherograms were stained for lipid with Oil Red O (Talluto *et al.*, 1958).

RESULTS AND DISCUSSION

Typical electropherograms obtained at different stages during the life cycle of coho salmon are presented in Fig. 1. No sex differences were apparent prior to gonadal development and the protein patterns from laboratory reared and wild yearling fish were similar and will not be discussed separately. Plasma from pre-smolting yearling coho contained 5 protein fractions as separated by the present methods. These fractions, in order of decreasing mobility have been designated 1, 2, 3, 4 and 5. Lipid staining was associated with fraction 2.

Many physiological changes occur concurrently during the smolt transformation (changes preceding and during migration from fresh to salt water—usually after one year in fresh water for coho). These changes have been discussed by Pickford and Atz (1957) and by Baggerman (1960). Carlson (personal communication, 1960) has demonstrated that fraction 1 is absent from smolting sockeye salmon plasma but that it reappears shortly after the fish enter salt water. Carlson's findings were extended to smolting coho salmon in this study.

Plasma electropherograms obtained from prepuberal 2-year-old coho which had spent a year in salt water were qualitatively similar to those obtained from pre-smolting yearlings. However, fraction 5 in all samples examined was more distinct at this stage and may be similar to γ-globulin in other vertebrates. Fraction 5 was the slowest moving component and was less concentrated, relative to the other fractions, in young fish. This increase may be related to increased antibody production as the fish is exposed to various organisms or it may be a normal development as the fish matures. Similar results have been obtained for γ-globulins in mammals (Moore *et al.*, 1945) and in birds (Brandt *et al.*, 1951; Vanstone *et al.*, 1955).

Other than a progressive increase in fraction 5, electropherograms of plasma obtained from maturing, spawning and spawned out 2½-year-old males were similar to those of immature fish of either sex. By contrast, with the onset of maturation in the female, a sixth plasma protein fraction appeared. This sixth fraction, fraction 6, which was also associated with lipid staining material, had the same mobility as fraction 4 and was admixed with it. The resulting mixed fraction was designated "4 + 6" in Fig. 1. Fraction 6 is probably a mixture of the lipovitellin and lipovitellenin complexes reported by McCully *et al.*, (1959)

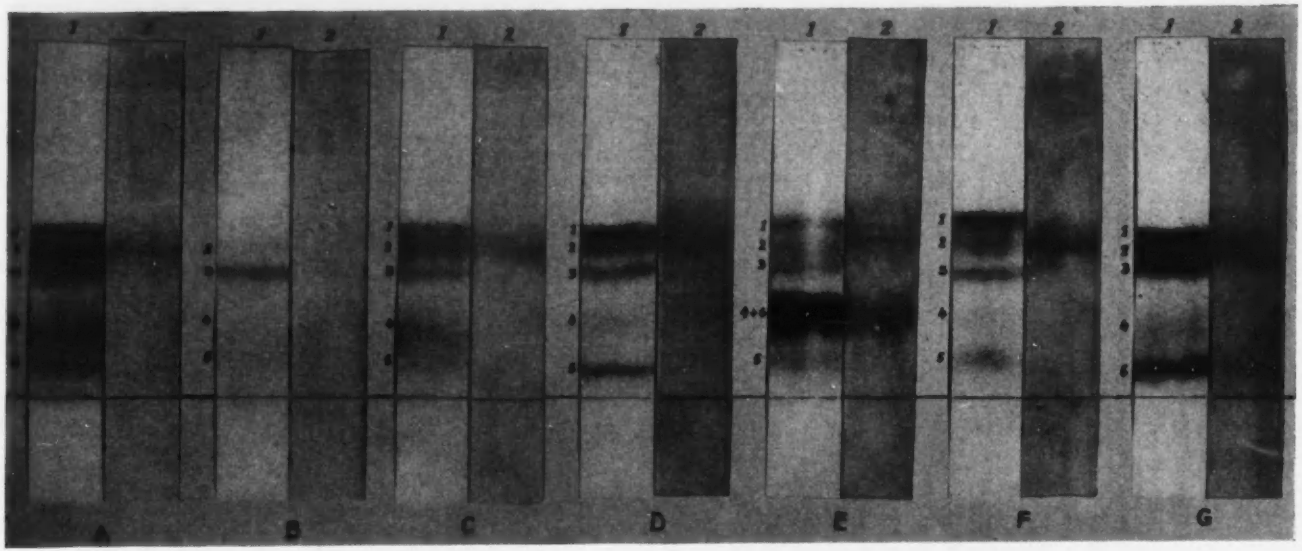

FIG. 1. Separation of plasma protein zones by electrophoresis with veronal buffer, pH 8.6, ionic strength 0.05, on Whatman 3MM paper. Temperature 5°C. Samples applied at pencil line. Stains (1) Azocarmine B (protein); (2) Oil Red O (lipid).

A. Pre-smolting yearling coho in fresh water.
B. Smolting yearling coho in fresh water.
C. Prepuberal 2-year-old coho in salt water.
D. Maturing 2½-year-old male coho in fresh water.
E. Maturing 2½-year-old female coho in fresh water.
F. Spawned-out male coho in fresh water.
G. Spawned-out female coho in fresh water.

in transit to the developing ova. This fraction, together with its associated lipid, disappeared from the plasma at about the same time that the eggs were released from the ovarian tissue. It was absent from spawning or spawned-out fish.

Laskowski (1936), working with female trout and tench, reported a similar decrease in serum vitellin coincident with maturation of the eggs. In addition Ridgway (personal communication, 1959) has found an antigenic component in maturing female sockeye sera and in sockeye eggs which was absent from male sera. His results might be explained by the *de novo* appearance of fraction 6 in maturing female plasma. These observations lend support to the theory that fraction 6 is indeed a yolk constituent, or more correctly, a mixture of yolk constituents including serum vitellin. In the bird these yolk components are produced in large part by the liver (Ranney and Chaikoff, 1951; Ranney *et al.*, 1951; Vanstone *et al.*, 1957). A similar site of synthesis may be postulated in the fish. These materials are then transported by the blood to the developing ova. At the completion of yolk development, but prior to spawning, the production of these constituents ceases with a resulting decline in their plasma levels.

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Influence of Size Upon the Adaptation of Steelhead Trout (*Salmo gairdneri*) and Chum Salmon (*Oncorhynchus keta*) to Sea Water[1,2]

By Arthur H. Houston[3]

ABSTRACT

Steelhead trout in the smolt phase of development adapted to sea water (salinity 22–24 parts per thousand) more rapidly and with less extensive departures from regulated conditions of water–electrolyte balance than did the larger post-smolts. By contrast, the extent and duration of the corresponding changes accompanying adaptation of juvenile chum salmon to sea water varied inversely with size. The data are discussed in relation to the distinction between smolting and non-smolting salmonid species.

INTRODUCTION

The anadromous Salmonidae appear to fall into two broad categories with respect to age-based variation in ability to survive transfer from fresh water into sea water. The first group of species, represented by *Oncorhynchus kisutch*, *Salmo salar*, *S. trutta* and *S. gairdneri*, include fish unable to resist sea water as underyearlings, but able to adapt to this environment at later stages (Huntsman and Hoar, 1939: Black, 1951; Parry, 1958). By contrast the second group, which includes *O. keta*, *O. gorbuscha* and possibly *O. nerka*, displays not only the ability to survive direct transfer into sea water in the fry stage (Black, 1951; Houston, 1959a) but also an active preference for this medium when given a choice between fresh and sea water (Houston, 1957; Baggerman, 1960a).

The development of ability to osmoregulate in sea water is correlated, in the first group of species, with the phenomenon of parr–smolt transformation. This process is accompanied by major variations in growth, metabolic activity and patterns of behaviour (Hoar, 1939, 1953, 1958, Baggerman, 1960b). Of particular interest from the viewpoint of sustained survival in sea water is the development at this time of the so-called "chloride secretory cells"; units believed to be the site of extrarenal electrolyte execretion in animals adapted to sea water (Hoar, 1951; Nishida, 1953; Parry, 1958). These observations together with data indicating marked decreases in plasma and tissue electrolyte levels of fish held in fresh water (Fontaine, 1951; Houston, 1959b, 1960) suggests that the animals at this stage undergo premigratory adaptation to the osmoregulatory requirements of marine life.

There is little evidence to suggest a comparable series of changes in the second group of species. Investigations by Black (1951) on the adaptation of

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chum salmon fry to sea water, by Hoar (1958) and Houston (1957) on the behaviour of chum, pink and sockeye fry, and by Skud (1955) on the length–weight relationship of juvenile pink salmon do not indicate premigratory changes equivalent to those seen in the smolting salmonids. The species of this second category are thought by Hoar (1958) to represent an evolutionary trend toward the abbreviation of the freshwater phase of life, and a loss of parr-smolt transformation.

While major variations in the sea water tolerance of parr and smolt stages of smolting species are well established, little attention has been directed toward possible variations in adaptive ability during the relatively short interval during which the outward characteristics of the smolt stage develop. The present study constitutes a preliminary investigation of this point in a typical smolting species, the steelhead trout (*Salmo gairdneri*). Estimates of adaptive ability have been based upon the time required for animals believed to represent the smolt and post-smolt stages to reduce the increases in plasma and tissue chloride levels which result from transfer into sea water. Because few small trout were available, no attempt has been made to assess the osmoregulatory capacities of the parr and silvery-parr stages. For purposes of comparison a similar investigation has been made of size-related variations in the ability of a typical non-smolting species, the chum salmon (*Oncorhynchus keta*), to adapt to sea water.

MATERIALS AND METHODS

STOCKS. Steelhead trout used in this investigation were obtained from the Smith's Falls (Cultus Lake) Hatchery of the British Columbia Game Commission. Chum salmon stocks were obtained through the courtesy of Dr Ferris Neave of the Fisheries Research Board of Canada Biological Station, Nanaimo, B.C. Both species were maintained at the University in running, dechlorinated water and were fed a commercial dry diet (Clark's Fish Food) supplemented with cod liver oil and yeast. Growth was good, and following the "delayed mortality" commonly encountered after transportation few deaths occurred in either group during the experimental period (May, June, July, 1957). Water temperature varied from 10 to 16°C over the period of maintenance and the fish were held under normal spring and summer conditions of light in the laboratory.

ACCLIMATION TO SEA WATER. Groups of 10 to 20 fish were acclimated to sea water in approximately 50-litre wooden boxes floated in constant temperature units and held at 10.0 ± 0.2°C. The fish were transferred in water from hatchery troughs to acclimation tanks and a period of 35 to 40 hours allowed for recovery from any shock of handling. The fresh water was then drained off to a depth of 8 cm and replaced with sea water from an overhead reservoir. Reservoir concentrations were adjusted so that a salinity of 22 to 24 parts per thousand was achieved in the acclimation tanks. Precaution was taken not to disturb the animals during transfer into the tanks, and during replacement of fresh water

with sea water, in order to avoid the initiation of "laboratory diuresis" and other conditions of electrolyte imbalance resulting from handling procedures (Meyer, 1948; Forster and Berglund, 1956).

SAMPLING. Steelhead trout were anesthetized prior to sampling in aqueous tricaine methanesulphonate (MS-222). Individual fish were loosely mounted ventral side uppermost upon a frame and blood samples drawn from the exposed bulbus arteriosus into heparinized and dried syringes. Muscle samples were excised from the dorsal fin area of the epaxial muscle band, rapidly dissected free of obvious fat, skin, cartilage and bone, and refrigerated until required in air-tight moist chambers.

Since chum salmon were too small to allow separate sampling of plasma and tissue, whole body chloride determinations were carried out. Fry were killed by a sharp blow on the head, rinsed twice in distilled water, lightly blotted with paper towelling and stored in the same manner as muscle samples.

ANALYTICAL PROCEDURES. Plasma chloride was estimated by the method of Schales and Schales (1941), and tissue chloride by the modified "open Carius" procedure proposed by Manery (1955). Details of these procedures have been described in an earlier publication (Houston, 1959b).

TREATMENT OF DATA. Changes in chloride concentration during adaptation to sea water have been related to the following variables: steelhead trout—weight, fork length, coefficient of condition; chum salmon—weight, fork length. Regression equations describing variations in plasma and tissue levels of chloride with each factor were calculated for fish adapted to fresh water, and for each acclimation period in sea water. Ability to regulate in sea water has been related to morphological characters believed to describe the smolt and post-smolt phases of development. Original determinations are tabulated in Houston (MS, 1958).

RESULTS

STEELHEAD TROUT

Assessment of steelhead trout as smolt or post-smolt has been based principally upon variations in length–weight relationship and changes in plasma chloride concentrations with weight. Figure 1 indicates a gradual decrease in the slope of the length-weight relationship with the most abrupt variation occurring between 35 and 45 g, and 16.5 to 17.5 cm. Within this range of lengths and weights, coefficients of condition varied from 0.55 to 0.75. In fish larger than 45 g the condition factor increased steadily with weight to a maximum of about 1.10 in fish heavier than 200 g. Too few data were available to allow precise estimates to be made of the range of condition factors in fish smaller than 30 g, but most values were greater than 0.7, suggesting that a decrease in condition accompanied parr–smolt transformation (followed by an increase when this was completed, at least under artificial conditions). In general the variations noted resembled those observed by Hoar (1939) for the weight, length and

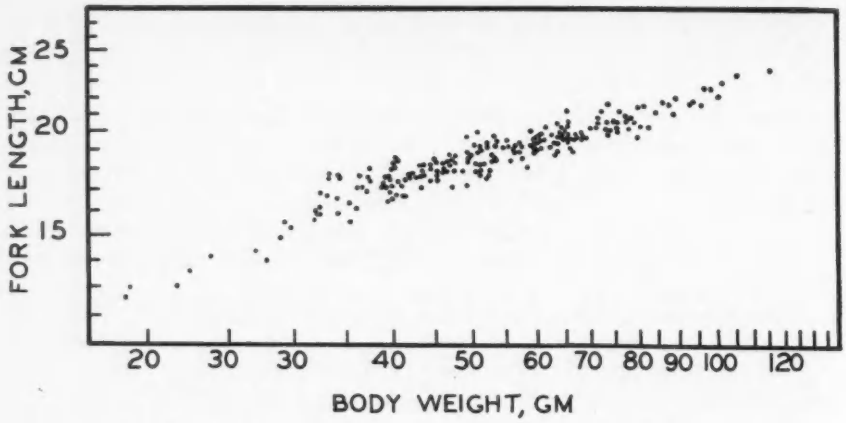

FIG. 1. Length–weight relationship of steelhead trout used in the experiments.

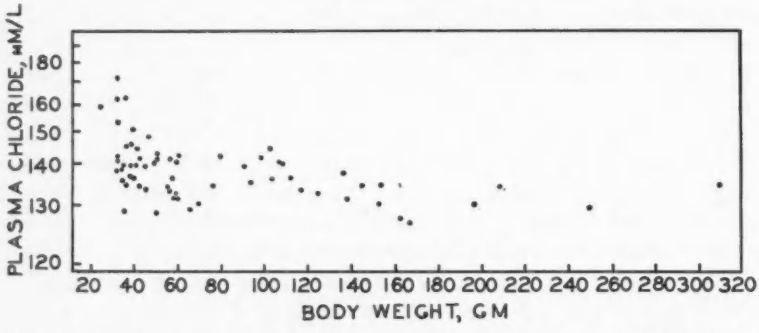

FIG. 2. Variation in plasma chloride concentration with weight in steelhead trout held in fresh water.

condition of Atlantic salmon, and by Kobayashi and Mogami (1958) for condition factor of rainbow trout (*S. irideus*).

In addition to the occurrence of a variation in the growth pattern, other data support the assumption that steelhead with the length, weight and condition-factor values characteristic of the inflection range were in the smolt phase of development. Figure 2 relates variations in plasma concentrations of chloride to weight in animals maintained in fresh water. Chloride levels decreased with increase in weight, with the most pronounced variations taking place between 40 and 55 g, or at about the same weight range within which the length-weight range changed most abruptly. Premigratory decreases in plasma and tissue electrolyte concentrations have been observed in several anadromous species (Fontaine, 1951; Kubo, 1955; Houston, 1960) and may be correlated with variations in blood levels of endocrine products related to electrolyte metabolism

(Fontaine and Hatey, 1954). It would appear reasonable to attribute these observations to the development and activity of "chloride-secretory cells", which is marked at about this stage of development in several migratory salmonids (Hoar, 1951; Nishida, 1953; Parry, 1958).

The studies of Maher and Larkin (1954) on the steelhead trout population of the Chilliwack River, British Columbia, also provide some support for the assumption. These authors found that downstream migrants of the same age-group (2 years) as those used in the present study had a mean fork length of 16.49 cm. It may be assumed that these animals were in the smolt stage of development. Reference to Fig. 1 indicates that this value is very close to the inflection point of the weight-length curve of the present steelhead population, and well within the range believed to be characteristic of the smolt phase of this group of trout.

On the basis of the foregoing data the following parameters have been taken as describing the smolt and post-smolt stages:

Smolt: fork length, 17 cm; weight, 40 gm; condition factor, 0.7.

Post-smolt: fork length, 20 cm; weight, 70 gm; condition factor, 0.9.

Tables I and II and Figs. 3 and 4 summarize changes in plasma and tissue levels of chloride computed for each of the above fish sizes, following transfer of the trout from fresh water into sea water. In each instance increases in chloride level accompanied transfer into sea water. Since fewer than 5% of the fish died during acclimation it may be assumed that the changes in internal electrolyte concentrations were within the tolerance limits of the species and constituted the adjustive phase of adaptation to sea water.

Increases in plasma chloride were consistently greater in fish believed to represent the post-smolt stage than in those assumed to be smolts. This was most apparent with respect to condition. Trout characterized by a coefficient of condition of 0.7 exhibited a maximum increase of 14.8%, while that in fish of condition factor 0.9 was 26.3% The maximum weight-related increase in plasma concentration in post-smolts was 29.4%, while that for length was 29.1%. Comparable values for smolts were 23.3 and 17.1% respectively.

The duration of increased plasma and tissue electrolyte levels and presumably of the adjustive phase of adaptation, was in each instance longer in post-smolts than in the smolts. At the final period of observation (168 hours), or before that time, the latter group displayed plasma concentrations lower than those observed in fresh water. At the same time post-smolt chloride levels were generally above the levels recorded in fish of similar size in fresh water. Extrapolation of the downward arms of the chloride–time curves suggested that these animals would require from 200 to 240 hours to achieve freshwater levels.

The degree and duration of deviations in plasma levels of chloride suggests, then, that steelhead in the post-smolt stage were less efficient in their adaptation to sea water than were those in the smolt stage.

TABLE I. Plasma chloride (pCl) changes in steelhead trout in sea water as related to weight, fork length, and coefficient of condition. In the regression equations, Y-values represent plasma chloride in millimoles per litre (mM/l); X-values represent respectively weight in grams (W), fork length in centimetres (FL), and coefficient of condition (C = 100 times weight in grams divided by the cube of fork length in centimetres).

A: Plasma Chloride versus Weight

Hours in sea water	Regression equation	W = 40 g (smolts)		W = 70 g (post-smolts)	
		pCl	% change	pCl	% change
0	Y = 152.8 − 0.3X	140.8		131.8	
4	Y = 159.8 − 0.2X	151.8	+ 7.8	145.8	+ 10.6
10	Y = 172.0 − 0.3X	160.0	+ 13.6	151.0	+ 14.6
15	Y = 172.7 − 0.2X	164.7	+ 17.0	158.7	+ 20.4
22	Y = 163.0 − 0.1X	159.0	+ 12.9	156.0	+ 18.4
36	Y = 177.6 − 0.1X	173.6	+ 23.3	170.6	+ 29.4
86	Y = 170.6 − 0.1X	166.6	+ 18.3	163.6	+ 24.1
126	Y = 147.9 + 0.03X	146.7	+ 4.2	145.8	+ 10.6
168	Y = 136.9 + 0.08X	133.7	− 5.0	131.3	− 0.4

B: Plasma Chloride versus Fork Length

Hours in sea water	Regression equation	FL = 17 cm (smolts)		FL = 20 cm (post-smolts)	
		pCl	% change	pCl	% change
0	Y = 187.1 − 2.6X	142.9		135.1	
4	Y = 155.9 − 0.5X	147.4	+ 3.2	145.9	+ 8.0
10	Y = 209.9 − 2.7X	164.0	+ 14.8	155.9	+ 15.4
15	Y = 238.0 − 4.3X	164.9	+ 15.4	152.0	+ 12.5
22	Y = 186.1 − 1.4X	162.3	+ 13.6	158.1	+ 17.0
36	Y = 108.4 + 3.3X	164.5	+ 15.1	174.4	+ 29.1
86	Y = 204.8 − 2.2X	167.4	+ 17.1	160.8	+ 19.0
126	Y = 157.7 − 0.3X	152.6	+ 6.9	151.7	+ 12.3
168	Y = 126.6 + 0.8X	140.2	− 1.8	142.6	+ 5.6

C: Plasma Chloride versus Coefficient of Condition

Hours in sea water	Regression equation	C = 0.7 (smolts)		C = 0.9 (post-smolts)	
		pCl	% change	pCl	% change
0	Y = 226.7 − 105.8X	152.6		131.5	
4	Y = 176.1 − 37.5X	149.8	− 1.8	142.3	+ 8.2
10	Y = 108.6 + 63.7X	153.2	+ 0.4	165.9	+ 26.2
15	Y = 78.1 + 97.8X	147.6	− 3.3	166.1	+ 26.3
22	Y = 157.5 + 2.3X	159.1	+ 4.3	159.6	+ 21.2
36	Y = 213.6 − 54.8X	175.2	+ 14.8	164.3	+ 24.9
86	Y = 159.7 + 4.6X	162.9	+ 6.7	163.8	+ 24.6
126	Y = 136.2 + 21.2X	151.0	− 1.0	155.3	+ 18.1
168	Y = 138.5 + 4.7X	141.8	− 7.1	142.7	+ 8.5

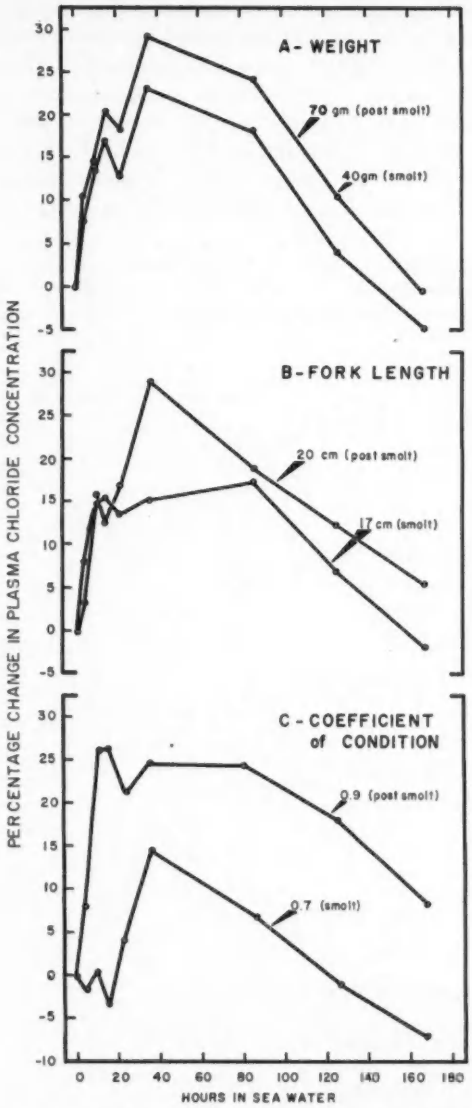

FIG. 3. Relation of percentage increase in plasma chloride (above the freshwater level) to length of exposure to sea water, for steelhead smolts and post-smolts as defined by three indices (weight, length and coefficient of condition).

Differences in tissue chloride levels between the two groups were equally conspicuous. While the duration of the period of raised chloride concentrations was about the same in smolts and post-smolts, the latter took up relatively more chloride than did the former. Since chloride ion is largely confined to the extracellular compartment and does not readily undergo osmotic inactivation by complex formation (Manery, 1954) it would appear likely that variations in tissue fluid distribution are relatively more marked in post-smolts, and that these fish underwent a greater degree of stress during adaptation than did smolts.

TABLE II. Tissue chloride (tCl) changes in steelhead trout in sea water as related to weight, fork length, and coefficient of condition. In the regression equations, Y-values represent tissue chloride in millimoles per kilogram of fresh tissue; X-values are as in Table I.

A: Tissue Chloride versus Weight

Hours in sea water	Regression equation	W = 40 g (smolts)		W = 70 g (post-smolts)	
		tCl	% change	tCl	% change
0	Y = 31.9 − 0.2X	23.9		17.9	
4	Y = 24.6 + 0.2X	32.6	36.4	38.6	115.6
10	Y = 44.1 − 0.2X	36.1	51.1	30.1	68.2
15	Y = 56.1 − 0.4X	40.1	67.8	28.1	57.0
22	Y = 37.8 − 0.01X	37.7	57.7	37.1	107.3
36	Y = 53.5 − 0.3X	41.5	73.6	32.5	81.6
86	Y = 30.3 − 0.1X	26.3	10.0	23.3	30.2
126	Y = 34.5 − 0.1X	30.5	27.6	27.5	53.6

B: Tissue Chloride versus Fork Length

Hours in sea water	Regression equation	FL = 17 cm (smolts)		FL = 20 cm (post-smolts)	
		tCl	% change	tCl	% change
0	Y = 48.7 − 1.4X	24.9		20.7	
4	Y = −21.2 + 2.9X	28.1	12.9	36.8	77.8
10	Y = 69.8 − 1.8X	39.2	57.4	33.8	63.3
15	Y = 64.1 − 1.5X	38.6	55.0	34.1	64.7
22	Y = 46.3 − 0.5X	37.8	51.8	36.3	75.4
36	Y = 96.5 − 3.0X	45.5	82.7	36.5	76.3
86	Y = 34.5 − 0.5X	26.0	4.4	24.5	18.4
126	Y = 15.0 + 0.7X	26.9	8.0	29.0	40.1

C: Tissue Chloride versus Coefficient of Condition

Hours in sea water	Regression equation	C = 0.7 (smolts)		C = 0.9 (post-smolts)	
		tCl	% change	tCl	% change
0	Y = 40.3 − 20.9X	25.7		21.5	
4	Y = 56.7 − 20.5X	42.3	64.6	38.2	77.8
10	Y = 49.7 − 19.6X	36.0	40.1	32.1	49.3
15	Y = 40.6 − 5.9X	36.5	42.0	35.3	64.2
22	Y = 24.7 + 15.9X	35.8	39.3	39.0	81.4
36	Y = 61.5 − 28.2X	41.8	62.7	36.1	67.9
86	Y = 28.5 − 4.4X	25.4	−1.2	24.5	14.0
126	Y = 62.8 − 46.2X			21.2	−1.4

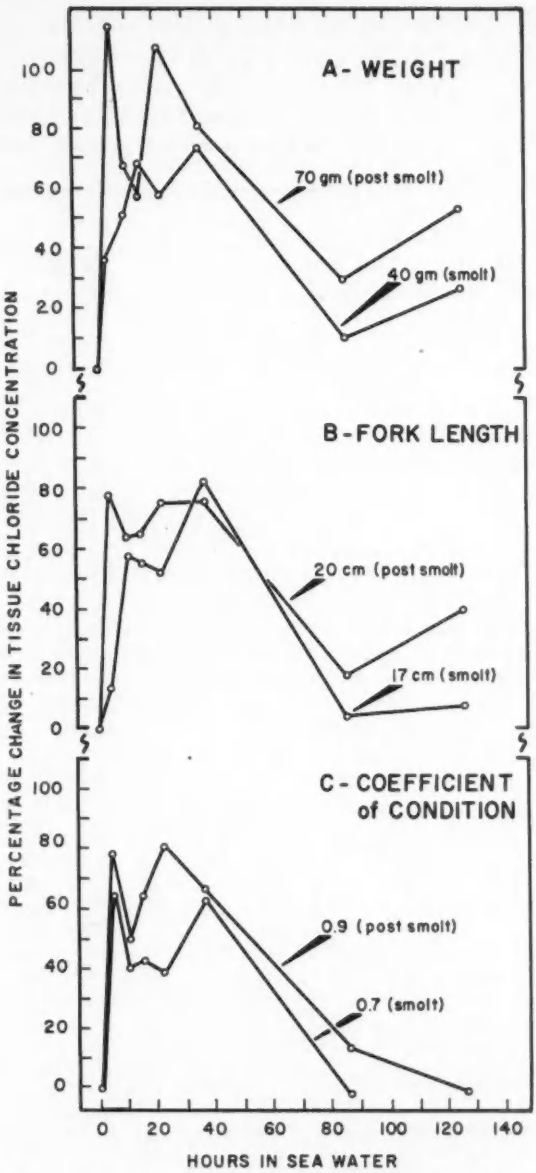

FIG. 4. Relation of percentage increase in tissue chloride (above the freshwater level) to length of exposure to sea water, for steelhead smolts and post-smolts as defined by three indices (weight, length and coefficient of condition).

Chum Salmon

Figure 5 indicates the variation of length with weight in chum salmon fry maintained in fresh water. Over the weight range of the population there was no indication of inflection, and no morphological basis for suggesting the occurrence of a transformation comparable to that seen in steelhead trout and the other

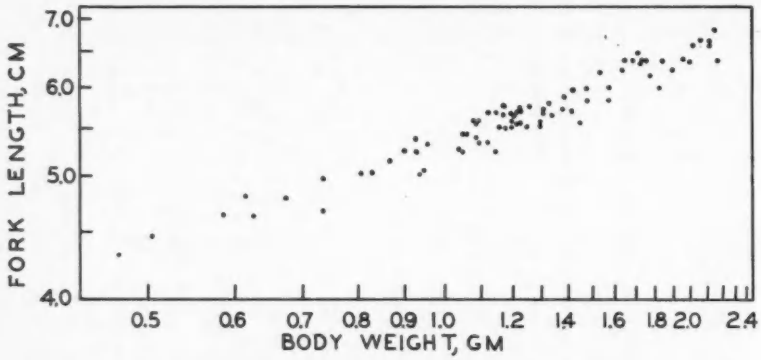

Fig. 5. Length–weight relationship of chum salmon fry used in the experiments.

Table III. Total body chloride (tot. Cl) changes in chum salmon fry in sea water as related to fork length and weight. In the regression equations, Y-values represent total body chloride in millimoles per kilogram of fresh tissue; X-values represent respectively fork length in millimetres (FL) and weight in grams (W).

A: Total Body Chloride versus Fork Length

Hours in sea water	Regression equation	FL = 50 mm		FL = 60 mm	
		tot. Cl	% change	tot. Cl	% change
0	Y = 110.3 – 0.6X	80.3		74.3	
3.5 – 4.5	Y = 262.6 – 2.9X	117.6	46.5	88.6	19.2
10–10.5	Y = 240.9 – 2.4X	120.9	50.6	96.9	30.4
14.5	Y = 278.5 – 2.8X	138.5	72.5	110.5	48.7
24	Y = 220.4 – 2.2X	110.4	37.5	88.4	19.0
37	Y = 229.3 – 2.3X	114.3	42.3	91.3	22.9

B: Total Body Chloride versus Weight

Hours in sea water	Regression equation	W = 0.9 g		W = 1.6 g	
		tot. Cl	% change	tot. Cl	% change
0	Y = 84.5 – 6.9X	78.3		73.5	
3.5–4.5	Y = 128.5 – 23.9X	107.0	36.7	90.3	22.9
10–10.5	Y = 149.1 – 33.0X	119.4	52.5	96.3	31.0
14.5	Y = 188.4 – 50.3X	143.1	82.8	107.9	46.8
24	Y = 138.1 – 37.4X	104.4	33.3	78.3	6.5
28	Y = 138.2 – 35.5X	106.2	35.6	81.4	10.8
37	Y = 140.8 – 34.5X	109.7	40.1	85.6	16.5

smolting species. A similar conclusion may be drawn from data provided by Skud (1955) on the pink salmon which also migrates within a few months after hatching. Other physiological, morphological and ethological data assembled by Hoar (1958) also lead to the conclusion that smolt transformation does not occur in chum and pink salmon.

Transfer from fresh water into sea water resulted in sharp changes in whole body levels of chloride (Table III, Fig. 6). As with steelhead, mortality was

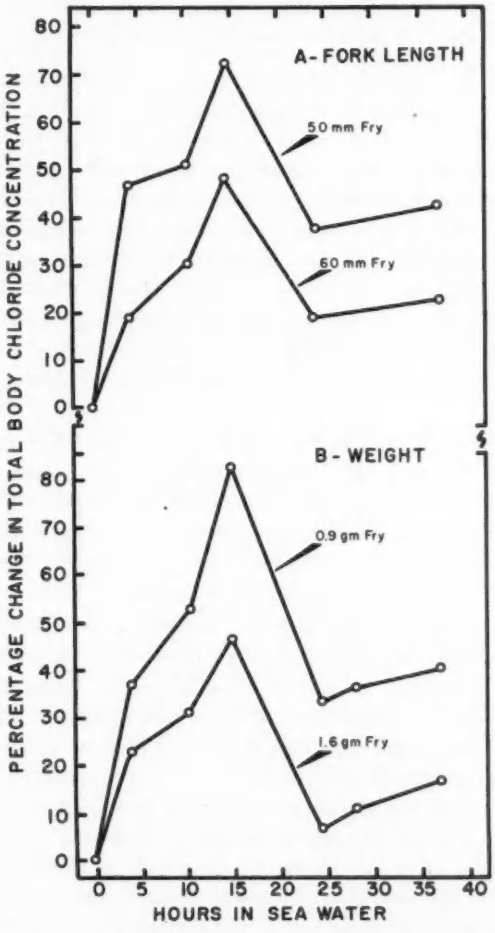

FIG. 6. Relation of percentage increase in total body chloride (above the freshwater level) to length of exposure to sea water, for chum salmon fry of two lengths.

slight during acclimation and changes in concentration are believed to reflect adjustive responses. The adjustive phase of adaptation lasted for 24 to 28 hours in these fish. A similar pattern of adaptation has been observed by Black (1951) for the same species.

In contrast to the situation observed in steelhead, increase in both fork length and weight was accompanied by a *decrease* in maximum uptake. Growth increases adaptive efficiency, indicating that some morphological relationship (e.g., exposed permeable surface area to body mass ratio) may play a greater role than a particular physiological condition, in the rapidity with which this species adapts to sea water.

DISCUSSION

While the general pattern of adaptation to sea water is comparable in both the steelhead trout and chum salmon, a major difference is seen in the influence of size. In the non-smolting species growth enhances osmoregulatory adaptability. This observation, together with data on changes in body chloride levels of freshwater forms, suggests that the juveniles while still in fresh water become increasingly preadapted to the osmoregulatory necessities of marine life. Presumably such preadaptation would include the development and activation of some extrarenal electrolyte excretory mechanism such as that postulated to exist in the gills.

The shift in osmotic adaptability appears to be unidirectional in both chum and pink salmon. In this laboratory chum salmon, and more noticeably pink salmon, have died if held in fresh water for longer than 7 or 8 months after hatching. Similarly Black (1951) observed that chum salmon maintained in freshwater underwent heavy mortality beginning in mid-August, accompanied by increases in water content and decreases in density. These observations and those of Ford (1958) on the numbers of glomeruli in the kidneys of freshwater-maintained pink salmon fry as compared to those of animals held in sea water suggest that death was due, at least in part, to loss of ability to regulate water and electrolyte levels. Although some workers have been able to hold pink salmon fry in fresh water for considerable periods of time (personal communication from W. E. Ricker), nevertheless there is some basis for assuming that movement into sea water is obligative for the non-smolting juvenile salmon.

In contrast to this situation in the non-smolting forms, many of the smolting species appear to be more facultative in their habitat requirements. In steelhead trout and in other species of this group seaward migration may occur after 2, 3, or even 4 years of freshwater life, and several of these species are represented by wholly freshwater varieties (e.g.: *O. nerka*, *S. salar*, *S. gairdneri*). While parr–smolt transformation results in the development of the regulatory systems required for marine osmoregulation, it does not necessarily result in the complete

loss of ability to survive in fresh water. The decreased adaptive efficiency of the post-smolts, however, suggests that adaptability to sea water varies on a seasonal basis. Corroborative evidence for this contention is available in studies indicating seasonal variations in the responses of sockeye and coho salmon fry and smolts to sea water (Houston, 1957; Hoar, 1958; Baggerman, 1960a), and in thyroid activity (Baggerman, 1960b). If this is the case, one might expect the occurrence of somewhat more complicated patterns of endocrinological function in the smolting forms. The resulting lability of the smolting species in regard to their osmotic habitat requirements may be a factor of definite survival value in the face of local conditions blocking downstream migration.

From the evolutionary point of view the observed variations in osmoregulatory ability with size, and inferences of obligative and facultative habitat requirements in the smolting and non-smolting species support the contention of Hoar (1958) that chum and pink salmon represent a branch of salmonid development which has diverged farther from the ancestral stock than has that represented by the present day smolting group of species.

From the more immediate viewpoint of the hatchery production of commercially desirable species such as the steelhead trout and Atlantic salmon, the data suggest that further investigation of the influence of size on adaptability to sea water would be desirable. Earlier investigations (Houston, 1959a) have shown that during adaptation to sea water the locomotor performance of salmonids is depressed. This effect is significantly correlated with the initial adjustive responses of the animals to sea water; increased plasma electrolyte concentrations, uptake and loss of cellular cations, and redistribution of tissue fluids. During this phase of locomotor depression the migrants may be more vulnerable to the action of predators. Thus release of hatchery fish at the weight and length producing most efficient adaptation to sea water should reduce both the extent and duration of adjustive changes and hence susceptibility to predation. Similarly, delays in downstream movement of either hatchery-reared or wild populations of salmonids by natural or artificial obstacles (e.g. power or other dams) are to be avoided wherever possible. Prolonged lack of feeding prior to entry into the sea, together with development of post-smolt changes in osmoregulatory mechanisms, may be expected to result in lack of efficiency in sea water, or the loss of ability to adapt successfully to the marine environment.

ACKNOWLEDGMENTS

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Comparison of Largest Great Slave Lake Fish With North American Records[1]

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ABSTRACT

The largest sizes for 11 of 25 species of Great Slave Lake fish are presented from material collected during studies dating from 1944 and other sources. The North American size records compiled from a literature search also are given. Only two of the Great Slave Lake records exceed the existing North American ones. It is suggested that the maximum size of fish is not attained among unexploited populations in oligotrophic lakes.

INTRODUCTION

Interest in the maximum size of fish is not restricted to anglers, because this information is contained in nearly all regional and general books on fish. The Great Slave Lake records for 11 of the 25 species in the Lake are presented and compared with the published North American records.

GREAT SLAVE LAKE RECORDS

A program has been carried out annually at Great Slave Lake by the Fisheries Research Board of Canada since 1944—see Keleher (1959) for a brief review and bibliography—and it is from these data that a compilation has been made. No systematic effort has been made previously to acquire such information, so it is possible that some fish taken by the commerical fishermen and others would have exceeded the present listings. The maximum size and other particulars are presented in Table I. Weight is the size criterion since most of the data are in this form. Records from other sources which exceed our own have been included, but we have in every instance listed the largest fish weighed by a Board employee.

The record for inconnu, *Stenodus leucichthys* (Güldenstädt), has been published (Rawson, 1947) and that for the longnose sucker, *Catostomus catostomus* (Forster), was provided by Dr R. H. D. Harris from his unpublished thesis.

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TABLE I. Records of the largest fish caught from Great Slave Lake. The footnotes indicate information not obtained by the Fisheries Research Board of Canada. See Kennedy, 1956, figure 6, for the lake divisions listed in the "Area" column.

Species	Weight		Fork length		Date	Area	Location	Caught by	Data from
	lb	*kg*	*in*	*mm*					
Lake whitefish	22.0	10.0	...	...	... 1958	H	Gros Cap	J. Kashak	Adolph Maerz[a]
" "	16.0	7.0	...	...	Aug. 14, 1950	M	Blanchet Is.	A. Kozial	J. A. Dick (FRB)
Round whitefish	4.5	2.0	20.0	508	Aug. 1, 1956	N	S. of Pearson Pt.	G. Whitley	H. Hamp (FRB)
Inconnu	55.0	25.0	...	...	... 1943?	A	Big Buffalo R.	Wm. Greer	Wm. Greer[b]
"	54.7	24.8	50.0	1270	Jan. 4, 1960	K	61° 41′ N, 112° 52′ W.	Wm. Janus	K. G. Roberts, C. G. Haight (FRB)
Lake trout	66.5	30.2	...	...	Winter, 1954	...	...	J. McCordic	G. Carter[c]
" "	66.0	29.9	...	...	Aug. 22, 1955	E	SE of Long Is.	R. Helmer	R. M. Hanson (FRB)
Arctic Grayling	5.0	2.3	...	...	Aug. 5, 1959	O	McKinley R. mouth	W. G. Clark	M. Ball[d]
" "	4.1	1.9	...	...	Aug. 14, 1958	H	Campbell Bay	D. Kirkness	H. Hamp (FRB)
Goldeye	2.6	1.2	...	...	July 1-4, 1959	A	Sulphur Pt.	...	F. Bastian (FRB)
Northern pike	30.0	13.6	...	...	Aug. 17, 1958	F	West Mirage Is.	C. Courtoreille	J. Dowling (FRB)
Longnose sucker	7.3	3.3	25.3	642	Aug. 8, 1951	D	Slave Pt.	Wm. Weselowski	R. H. D. Harris (FRB)
White sucker	6.7	3.0	...	...	Dec. 8, 1954	F	10 miles NW Ptarmigan Pt.	D. C. Scott	D. C. Scott (FRB)
Burbot	18.5	8.4	36.9	937	July 6, 1956	E	Redrock Pt.	M. Fedorus	J. Sunley (FRB)
Walleye	11.0	5.0	30.7	780	July 5, 1956	B	20 miles SE Outpost Is.	Moneias Bros.	G. Anderson (FRB)

[a]Foreman, McInnes Products Corporation.
[b]Resident, Big Buffalo River; personal communication to Dr D. S. Rawson.
[c]Carter Fisheries Ltd.; specimen in possession of R. W. Park, Park-Hannesson Ltd., Winnipeg.
[d]Fishing contest editor, *Field and Stream*.

NORTH AMERICAN RECORDS

With the exception of the angling contest records started in 1911 by *Field and Stream* (Anon., 1960), maximum sizes of North American freshwater fish only can be found by a search of the literature. Our search probably has not been complete, but presentation of the results may encourage other contributions to this subject.

The maximum size reported for a lake whitefish, *Coregonus clupeaformis* (Mitchill), is 42 lb. According to Louis P. Hogstad of Duluth, Minnesota, it was caught about 1918 at Isle Royale in Lake Superior (Van Oosten, 1946). No other records exceeding 26 lb have been located. Koelz (1929) and Van Oosten (1946) mentioned reports of lake whitefish attaining this size in the Great Lakes. The report (Anon., 1946; Derback, 1947) of a 26 lb lake whitefish being taken in Manitoba during the 1923–24 fishing season should no longer be accepted. This fish, captured in Sturgeon Bay, Lake Winnipeg, actually weighed 24 lb (G. E. Butler, personal communication, 1960).

The round whitefish, *Prosopium cylindraceum* (Pallas), is considerably smaller than the lake whitefish. The maximum reported has been about 5 lb for a Lake Superior fish (Koelz, 1929).

Wynne-Edwards (1952) pointed out that inconnu attain a larger size in the USSR, where a weight of 88 lb (40 kg) has been recorded, than they do in North America. Here the two heaviest fish have been "just over 63" lb and 56.5 lb (Dymond, 1943).

With respect to the size of lake trout, *Cristivomer namaycush* (Walbaum), Dr S. Mitchill stated at Michilimackinac in Lake Huron one was known to attain 120 lb (Richardson, 1836). Other credible records are much less. An 88 lb lake trout was caught at Grand Haven, Michigan, in 1864 (Goode, 1884). In 1906 an 87 lb lake trout was taken on rod and reel from Lake Bennett, Yukon Territory (Anon., 1953). Mr T. Goodman of Fairford, Manitoba, netted an 80.5 lb fish in Lake Athabasca on September 11, 1955. It was photographed (Symington, 1958) and is now on display at the Saskatchewan Museum of Natural History, Regina. The angling record is a Lake Superior fish of 63.1 lb, caught in 1952.

Prior to 1959 about 4 lb appeared to be the North American limit for Arctic grayling, *Thymallus arcticus* (Pallas). The fishing contest editor for *Field and Stream* informs me (M. Ball, personal communication, 1960) that a new angling record of 5.0 lb has been accepted from Great Slave Lake (Table I). The previous angling record, dating from 1955, was 4 lb from the Clearwater River, Saskatchewan. Manchester (1954) stated that the largest grayling on record, weighing "over four pounds", came from Wrigley Harbour, Great Slave Lake. However, he has advised me (personal communication, 1959) that this statement cannot now be substantiated.

The upper size limit of the goldeye, *Amphiodon alosoides* Rafinesque, is about 3 lb. The largest specimen cited by Trautman (1957) was a 3.1 lb female. One caught in the North Platte River, Wyoming, weighed 2.7 lb (Simon, 1946).

Northern pike, *Esox lucius* Linnaeus, from North America appear not to exceed 50 lb. Chambers (1896) saw a 49 lb pike from Lac Tschotagama, Quebec, in 1890. The *Field and Stream* angling record is 46.1 lb for one taken from a New York reservoir in 1940.

Neither the longnose sucker nor the white sucker, *Catostomus commersoni* (Lacèpéde), attain a large size. Longnose suckers weighing 5 lb were "not uncommon" in Lake Abitibi in 1925 (Dymond and Hart, 1927), but the 7.3 lb specimen from Great Slave Lake is apparently the record. White suckers previously had seemed the larger species: Webster (1942) reported a 6.9 lb white sucker from East Twin Lake, Connecticut, in 1939. A 6.5 lb white sucker was caught in Maine in 1957 (Everhart, 1958).

The burbot, *Lota lota* (Linnaeus), can attain a weight of 75 lb according to Wynne-Edwards (1952). The record for North America presumably is Dall's (1870) listing of 60 lb for Alaska.

Most authors report 25 lb as the maximum for the walleye, *Stizostedion vitreum* (Mitchill), probably on the basis of Goode's (1884) statement. An Associated Press news release stated that a 25 lb walleye was caught on August 2, 1960, at Old Hickory Lake, Tennessee, by a Nashville angler, Marby Harper. If substantiated, this would be the new angling record.[2] The existing one is for a 22.2 lb fish caught at Fort Erie, Ontario, in 1943. In 1950 a 23.6 lb walleye was caught in the Moon River, Ontario (Scott, 1954).

DISCUSSION

With the exception of the Arctic grayling and longnose sucker, none of the records for Great Slave Lake fish exceed in weight those caught elsewhere. The round whitefish and white sucker records are near the maximum reported but the remainder are much less. Of the present Lake records, none were fish taken during the Board's general survey from 1944 to 1947, when about 12,000 fish were examined (Rawson, 1951). This suggests that fish do not attain their maximum size under these conditions—that is, among relatively unexploited fish populations in an oligotrophic lake. The *Field and Stream* article (Anon., 1960) substantiates this conclusion.

Two of the North American records cited seem doubtful. These are 42 lb for lake whitefish and 120 lb for lake trout. The disparity between the former and the other known records of 26 lb is too great to be accepted on the evidence presented. Likewise, the 120 lb lake trout far exceeds any other known. While some authors may accept 120 lb lake trout, it is felt this century-old record should be challenged.

ACKNOWLEDGMENTS

While assistance has been received from many individuals, particular acknowledgment should be made to my associate, Mr N. H. F. Watson and to Dr J. Van Oosten.

[2]This record is now accepted by *Field and Stream*; see the issue of March, 1961, vol. 55, No. 11, p. 71.

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The Lake Trout of Lac la Ronge, Saskatchewan[1]

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CONTENTS

ABSTRACT

The lake trout, *Salvelinus namaycush*, of Lac la Ronge was studied in the years 1948 to 1959 using gill netting, tagging, creel census and sampling of the spawning run. The trout spawned on shallow rocky reefs in the first week of October, when water temperatures were about 10°C (50°F). Of the spawning run, 82% were from 7 to 12 years old. Possibly 10% of the mature trout failed to spawn in any single year.

Growth rate varied widely in individuals and the average rate produced, at 10 years, a trout of 26 inches (66 cm) fork length and weighing 8 lb (3.6 kg). No difference was found between growth rates of males and females and there was no change in average growth rate during the 10-year study. However, trout in the main lake grew considerably faster during their first 5 years than those in the deeper, colder Hunter Bay. Thirty-three trout weighing from 30 to 43 lb were examined.

Ciscoes, whitefish, ninespine sticklebacks and other fish made up 90% of the food of adult trout. The crustaceans *Mysis* and *Pontoporeia* were eaten in moderate quantities, especially by trout in their third and fourth years. Two cestode parasites were found in the intestine and the larvae of a third in the muscle of trout.

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The trout were widely scattered from break-up of ice in mid-May to late June. As the upper water warmed above 10°C (50°F) they moved down and were concentrated in areas deeper than 20 m (65 ft) during July and August. As the water cooled they again spread into shallow water about mid-September. Of 429 tagged, 60 or 14% were recovered during the first, second and third years after tagging. Recoveries showed extensive movement throughout the lake and a moderate exchange between the main lake and Hunter Bay.

In standard gill-net catches plankton-feeding and bottom-feeding fish outweighed piscivores by 3 to 1 in the main lake and 1.8 to 1 in Hunter Bay. Catch data suggest that the trout population was greater in 1958–59 than in 1948–49. The average size of trout caught and the year-class composition were unchanged after 10 years.

By diverting commercial fishing from trout to whitefish the average commercial catch in the 10-year period has been maintained at 250,000 lb per year, as compared to 316,000 lb in the previous 30 years. The anglers' catch of trout now averages 30,000 lb per year and this could be doubled without exceeding the known capacity of the lake for trout production. The creel census shows no decrease in average size of trout caught, average catch per angler, and number of very large trout taken.

INTRODUCTION

THE LAKE TROUT, *Salvelinus* (*Cristivomer*) *namaycush*, is a highly regarded game fish in lakes of northern Saskatchewan. It is also one of the main commercial species, ranking second only to the whitefish in the value of its annual production. It was desirable, therefore, to obtain detailed knowledge of the life history and ecology of this important species in Saskatchewan lakes.

Lac la Ronge was fished commercially and known as a good "trout" lake long before the road made it possible for several thousand anglers to visit it annually. It is probably unique among lakes of this latitude in western Canada in having certain conditions which allow anglers to catch trout readily throughout the summer. The possibility of finding the reasons for this situation added to the scientific interest of the present investigation.

In the 10 years 1948 to 1957, the lake trout were sampled and studied as a part of the program of fisheries research and management on Lac la Ronge. In 1958 and 1959 a more intensive study was carried on, aimed particularly at the seasonal distribution and ecology of the lake trout population. In all years, advantage was taken of the heavy angling for lake trout as revealed by a full-time creel census.

Lac la Ronge, Fig. 1, is a lake of 550 square miles (1425 km²) lying across the southern margin of the Precambrian Shield, and just south of the Churchill River at Latitude 55°. Its general limnology and fisheries were described by Rawson and Atton (1953). By comparison with other large lakes in Saskatchewan Lac la Ronge has been shown to be moderately productive, richer than lakes on the shield but poorer than those on the glacial drift to the south. Lac la Ronge proper can be described as mesotrophic, but Hunter Bay, a 50 square mile part on the east side, is distinctly oligotrophic (Rawson, 1960). Previous studies of the fish of Lac la Ronge include an investigation of the walleye (Rawson, 1957) and the whitefish (Qadri, 1961). The present study continues a plan to investigate each of the major fish species in the lake.

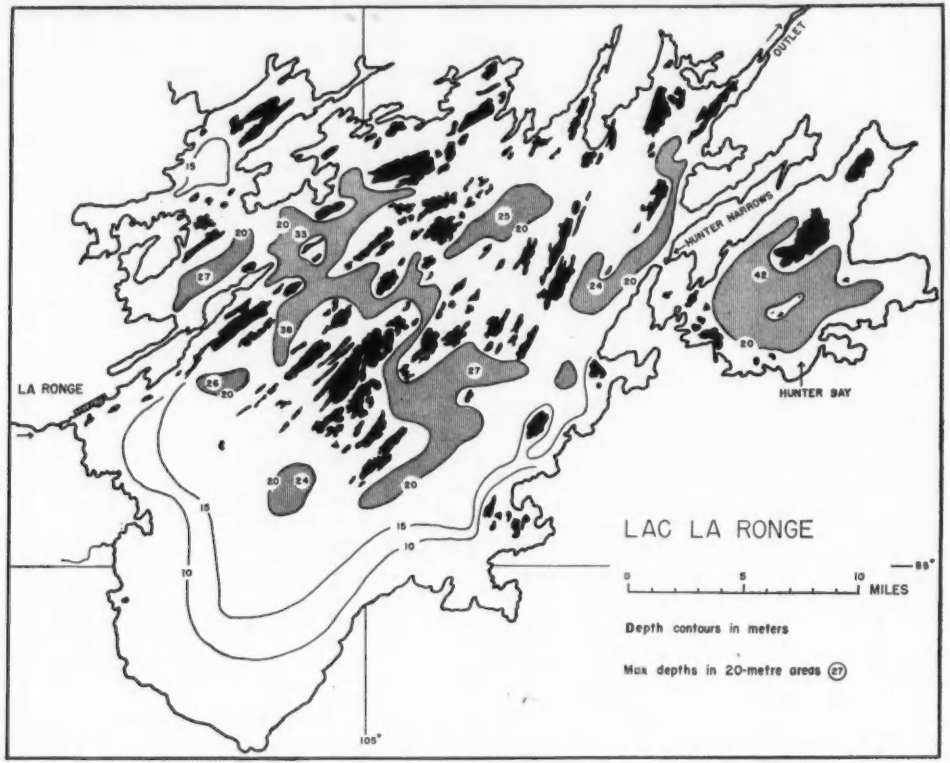

FIG. 1. Map of Lac la Ronge showing the islands, in black, and the areas inside the 20-metre (65 ft) contour, shaded. Lake trout are concentrated in these areas during July and August. For detailed soundings of Lac la Ronge see Chart 6281, Canadian Hydrographic Service, Ottawa.

SPECIFIC IDENTITY AND GEOGRAPHIC DISTRIBUTION

The lake trout is widely distributed across northern North America from Alaska to the Maritime Provinces and from the Great Lakes to some of the Arctic Islands. Unlike many of our northern fish species it has no close relative in Siberia. Walters (1955) and others have suggested that the lake trout entered our northern waters in recent times, coming from a refugium in the Upper Mississippi Valley. Although widespread, the lake trout is not, as stated by Slastenenko (1958), found in "all lakes of sufficient depth to provide cold water in summer".

The occurrence of lake trout in Saskatchewan is restricted to its northern half. It is found in most of the deep lakes on the Precambrian Shield and north of Latitude 55°. It occurs in only a few of the lakes between 54 and 55°. These include Cold Lake on the west boundary, four lakes in the Prince Albert National Park (Rawson, 1936) and East Trout Lake, all of which drain north into the Churchill River. Farther to the east, trout are found in Little Bear, Deschambault, Jan, Hansen and Amisk Lakes, all draining into the Saskatchewan River. Lac la Ronge, lying across the margin of the Precambrian and cut by Latitude 55°, may be regarded as near the southern edge of the typical lake trout area.

In spite of its wide distribution the lake trout appears to be a rather homogeneous species, providing little justification for the recognition of subspecies, with the exception of the siscowet of Lake Superior. The trout of Lac la Ronge are large and well nourished (Fig. 2). Growth studies, reported in a later section, will show that while growth in length of Lac la Ronge trout is much like that of trout in Great Slave Lake, the older fish in Lac la Ronge are considerably heavier than those in Great Slave Lake. It was also found that trout in small lakes near Lac la Ronge often differ considerably in body proportions. The head lengths of adult Lac la Ronge trout average one-fifth of the body (fork) length while trout in MacKay Lake, about 10 miles north of La Ronge, have heads averaging one-quarter of the body length (Fig. 2).

The external colour of Lac la Ronge trout is typically blue-grey to blue-black with occasionally a greenish or brownish cast. The light-coloured spots usually cover an area of 3 to 7 scales. They do not show the light pink or reddish tint often mentioned in descriptions of this species. In some trout, such as the specimen from MacKay Lake shown in Fig. 2, the white spots are large in relation to the dark pigment, thus giving the appearance of a dark reticulum on a light background. The paired and anal fins exhibit varying shades of red and are sometimes quite vivid in colour, contrasting with their white leading edges. The belly is usually silvery or creamy white.

The flesh colour of Lac la Ronge trout ranges from pale creamy white, through light yellow to orange and red. The flesh of immature fish, less than about 17 inches (43 cm) in length, is almost always pale cream or white. Pale shades predominate in adult fish but some individuals exhibit a striking orange or reddish colour. In a preliminary attempt at objective recording of flesh colour, samples of all available flesh colours were collected and frozen. An artist was engaged

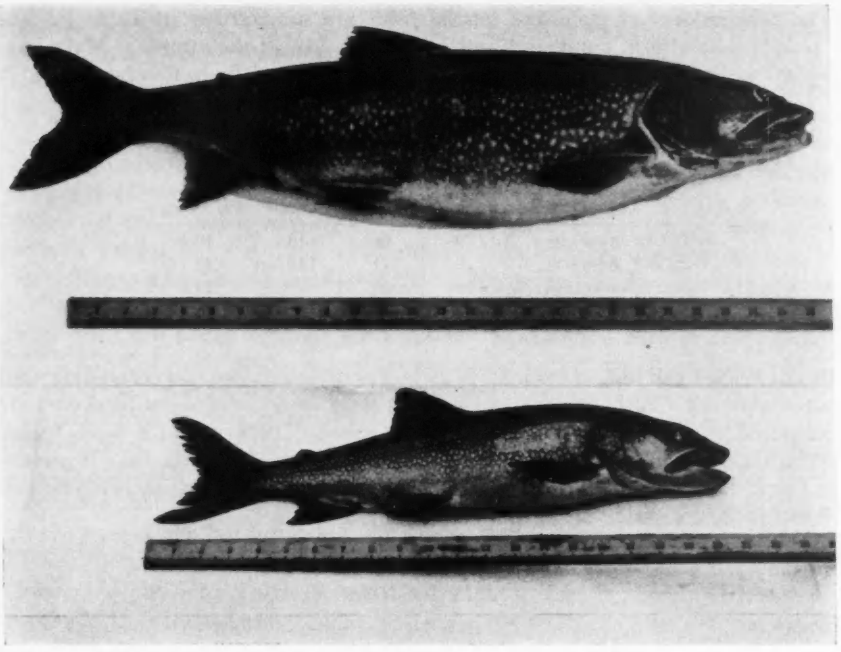

FIG. 2. *Above:* Typical lake trout from Lac la Ronge, fork length 27 inches (68.5 cm), weight 9.3 lb (4.2 kg), and age 11 years. *Below:* Large-headed trout from MacKay Lake, fork length 19.3 inches (49 cm), weight 2.9 lb (1.3 kg), and age 8 years. A Lac la Ronge trout of 8 years would be 20% longer and more than double the weight of the MacKay Lake specimen.

to prepare a matching series of colour sheets which were subdivided to provide each worker with a standard set. The five selected standards were described as (1) Creamy white (2) Pale yellow (3) Yellow orange (4) Bright orange. A fifth standard, Orange red, was prepared to match the bright flesh colour of trout from adjacent lakes. However none of the trout examined from Lac la Ronge showed this degree of colour. The approximate rating of our colour standards on the Munsell system of notation was as follows:

1. 3.5 Y 8.6/5.5
2. 9 YR 7.7/6.8
3. 6.5 YR 7.9/9.8
4. 3.5 YR 6.5/11
5. 2.2 YR 6.2/9.2

In the period June 20 to September 15, 1960, flesh colour was recorded for 337 trout taken by anglers on Lac la Ronge. Length, sex and locality was recorded and egg samples were taken from the ovaries of most of the females.

The flesh colours of male and female trout are summarized in Table I. The "pale" coloured fish, numbers 1 and 2, made up 83% of the sample. Males were more numerous among the 17% with "bright" coloured flesh, numbers 3 and 4.

TABLE I. Flesh colour of 337 lake trout 18–31 inches (45.6–78.6 cm) fork length, from Lac la Ronge, June to September, 1960.

Colour	Females	Males	Total	Percentage
1. Creamy white	70	66	136	40.4
2. Pale yellow	73	71	144	42.7
3. Yellow orange	18	28	46	13.6
4. Bright orange	3	8	11	3.3

Bright-fleshed fish ranged from 19 to 29 inches in length, thus no correlation with size was found. Although the two sets of colour standards cannot be accurately compared, it is apparent that Miller and Kennedy (1948) found a much higher percentage of trout with bright-coloured flesh in Great Bear Lake than is present in Lac la Ronge. The possible relation of flesh colour to spawning is referred to below in the discussion of spawning and egg diameter.

LIFE HISTORY

SPAWNING

The lake trout in Lac la Ronge usually spawn in the first week of October at depths of 1 to 3 metres (3–10 ft) on stony or rocky reefs. Since most of the lake is characterized by rocky shores and islands there is an abundance of bottom suitable for spawning. In several years lake trout eggs have been collected for hatchery purposes on reefs south of Bear Island in the north central part of the lake. These operations have made it possible to obtain detailed observations on the spawning trout.

The spawning run usually begins to come in on the reefs about September 25 but few ripe males or females are found before October 1. The males showed a slight tendency to precede the females in reaching the spawning area and becoming ready to spawn., In five years, the periods at which trout spawn was taken near Bear Island are as follows:

1949, October 3–9, a few eggs taken September 28
1950, October 4–11
1952, October 1–3
1953, October 2–6
1959, October 4–9

Thus the trout of Lac la Ronge appear to spawn in a well defined and rather short period which usually lies within the first week of October. Restriction of the spawning period to a week or less appears to be somewhat uncommon. In the Great Lakes, Van Oosten (1935) indicates spawning periods of more than one month. However, McCrimmon (1958) indicates that trout spawning in Lake

Simcoe, Ontario, was mostly completed in 7 to 9 days and Royce (1951) reports that in Lake George, New York, it is completed in 7 to 10 days. In the Great Lakes and in eastern Canada spawning is often in late October or November. In Great Slave Lake (latitude 62°) spawning takes place in mid-September and in Great Bear Lake (latitude 66°) spawning begins in mid-August (Miller and Kennedy, 1948). The number of spawning fish handled varied in the five years from 320 to 650. In four years in which records are available the males outnumbered the females, making up 66, 57, 58 and 55 percent, an average sex ratio of 1:0.7. In 1950, 1952, 1958 and 1959 the opportunity was taken to tag and release a considerable number of the trout captured on the spawning grounds.

Water temperatures of about 13° C (55.4° F) were common in late September when the trout began to arrive on the reefs. Spawning usually started at about 11° C (51.8° F) and at the end of the spawning period water temperatures of 9 to 10° C (48.2–50° F) were commonly observed. Spawning of lake trout in water temperatures of approximately 10 to 12° C (50–53.6° F) has been reported by many investigators. Royce (1951) reported that in some lakes in New York State, spawning occurs about the time of autumn circulation. McCrimmon (1958) also notes that lake trout in Lake Simcoe, Ontario, come to the spawning grounds at the time of the fall turnover. At Lac la Ronge the autumn turnover usually occurs in late August or early September. Thus the spawning temperatures referred to above for Lac la Ronge are not just surface temperatures but extend to the level of the spawning beds and deeper.

The age at which the trout of Lac la Ronge reach sexual maturity ranges from 5 to 8 or 9 years. A very few trout were mature at 5 years, nearly one-third at 6 years, two-thirds at 7 and almost all at 8. The correlation of length with sexual maturity was somewhat closer than that with age. Thus only the largest of the 6-year-olds were mature and any fish more than 20 inches (51 cm) in length and weighing more than 3 lb (1.4 kg) was almost certain to be mature although the age of a 20-inch trout might be anywhere from 5 to 9 years. Lake trout in the New York lakes (Royce, 1951) and in small lakes in Ontario (Martin, 1952) mature somewhat earlier. Those in Great Slave Lake mature a year or two later (Kennedy, 1954) and those in Great Bear Lake not until their 13th year when they are still only 16.5 inches (42 cm) long and 2 lb (0.9 kg) in weight (Miller and Kennedy, 1948).

The fork length distribution of the spawning run was determined from two samples, 145 measured in 1958 and 233 in 1959. Since both samples have the same range and mode they have been put together. The combined distribution arranged by mid-class lengths in inches is as follows:

Length	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
No. of trout	8	14	30	63	75	67	54	32	21	7	1	2	2	0	1	0	1

The modal length of 24 inches (61 cm) corresponds to the average length of an 8-year-old-trout. Reading the presumed ages of fish of other length from the growth curve (Fig. 3 below) it can be deduced that 74% of the spawning

run was made up of trout from 7 to 10 years of age and 82% from 7 to 12 years of age. Examination of the scales of a small sample of fish from the spawning run confirmed this deduction. They were from 7 to 12 years old with the largest number in the 8-year class.

It is known that the lake trout in some northern waters do not spawn every year. Thus trout in Great Bear Lake spawn about once in three years (Miller and Kennedy, 1948), those in Great Slave Lake usually in alternate years (Kennedy, 1954). Cuerrier and Schultz (1957) suggest that the lake trout of Waterton Lake, in southwestern Alberta, spawn in alternate years up to 10 years and annually thereafter. Observations at Lac la Ronge suggested that some of the mature female trout did not spawn every year. Samples of trout ovaries were taken from June to September, 1960, to determine the rate of egg growth. From each trout a sample of about 5 cc of eggs was taken, together with date, locality and fork length. Flesh colour was recorded for 164 of the 228 fish examined. The egg samples were preserved in 10% formalin for later measurement.

The average diameter of formalin-hardened trout eggs increased from 2.8 mm in late June to 6.1 mm at spawning time, October 5 and 6. The following averages are based on the measurement of 228 samples, a sample being 10 eggs from each fish. In the period August 16 to 31 the samples were too few to justify calculation.

June 20–30	average 2.8 mm	August 1–15	average 4.7 mm
July 1–15	" 3.5 mm	September 1–15	average 5.8 mm
July 15–30	" 4.1 mm	October 5–6 (spawning)	6.1 mm

These egg sizes are not unlike those reported by Dymond (1928) who found that in late August, 1927, the larger lake trout in western Lake Ontario had eggs from 4 to 5 mm in diameter.

Among the 228 female trout from which samples were taken, 18 had "undersized" eggs. These ranged from 1.6 to 2.5 mm diameter and were never more than 60% of the average diameter of "normal" eggs taken on the same date. These fish ranged from 20 to 31 inches in length and were apparently sexually mature but not preparing to spawn in the current season. This group of non-spawners represents only 8% of the sample, a much smaller proportion than those mentioned above for trout in Great Slave and Great Bear Lakes. Of these 18 non-spawners 7 had bright flesh colour, a marked contrast with the spawning females, almost all of which were pale. Miller and Kennedy (1948) found an even higher proportion of bright-fleshed individuals in the non-spawning trout of Great Slave Lake.

Rate of Growth

Information for the growth study comes from the scales of more than 900 trout for which fork length, weight and sex were also recorded. Most of these fish were taken in "standard" gangs of gill net described in a later section. A large sample was taken in the first 2 years of investigation, 1948 to 1949, and a

second large sample 10 years later, in 1958. The distribution of these samples was as follows:

	Main lake	Hunter Bay
1948	178	22
1949	109	123
1953	43	59
1958	277	113
Totals	607	317

Three or more scales from each fish were impressed on cellulose acetate slides with a jeweller's roller and read by projection at a suitable magnification. Most of the scales were examined independently by two experienced readers who agreed on about 90% of the interpretations. Twelve of the 924 readings have been rejected as representing distinctly abnormal conditions. Helpful advice on scale interpretation was received from the experienced readers trained by Dr W. A. Kennedy of the Fisheries Research Board of Canada Biological Station, London, Ont.

The growth analysis of the Lac la Ronge trout was designed to determine the general growth rate and whether there was (a) any sex difference, (b) any difference between growth of trout in Hunter Bay and the main lake, (c) any change in growth rate over the 10-year period of study.

By determining separate average lengths for males and females of each age group and plotting these as two curves it was shown that there was no detectable difference in the growth rate of the two sexes. This was true for the whole samples from the main lake and from Hunter Bay and also for the largest subsample of 277 trout from the main lake, in 1958.

A comparison was made of the average lengths of trout of each age group from the main lake in 1948–49 and those taken in 1958. The average lengths of the 1958 fish were consistently greater than those of 1948–49. In the year-classes 7 to 11, in which sample numbers ranged from 52 to 108, the comparable average lengths are as follows:

	1948–49	1958
7 years	22.9	23.4
8 years	23.7	24.4
9 years	24.9	25.8
10 years	25.7	26.5
11 years	26.9	27.6

Since the trout caught in 1958 in the main part of Lac la Ronge averaged nearly 3% longer than those caught in 1948–49 it would appear that a slight increase in rate of growth may have occurred in this period. The sample is not large enough to place great reliance on this determination. A similar analysis of the trout caught in Hunter Bay showed no evidence of change in the rate of growth over the 10-year period.

A preliminary examination revealed a difference in the growth pattern of trout in the main part of Lac la Ronge and in Hunter Bay. Thus the age and length data for trout from the two areas are shown separately in Tables II and III and two curves appear on the graph Fig. 3. An impressive feature of these data

TABLE II. The numbers of lake trout of given fork lengths in a sample of 601 fish representing age groups 2 to 18 years from the main part of Lac la Ronge 1948 to 1958. Lengths were measured to tenths of inches and are listed in half-inch groups; thus 7.2 includes 7.0 to 7.4, 7.7 includes 7.5 to 7.9 etc.

Mid-class length inches	Age in years																
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
7.2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
7.7	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
8.2	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
8.7	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
9.2	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
9.7	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
10.2	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
10.7	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
11.2	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
11.7	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
12.2	0	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
12.7	2	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...
13.2	1	1	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...
13.7	...	2	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...
14.2	...	3	0	...	0	...	...	...	...	...	...	...	...	...	...	...	...
14.7	...	0	0	...	0	...	...	...	...	...	...	...	...	...	...	...	...
15.2	...	1	0	...	0	...	...	...	...	...	...	...	...	...	...	...	...
15.7	...	3	0	...	0	...	...	...	...	...	...	...	...	...	...	...	...
16.2	...	2	1	...	2	...	...	...	...	...	...	...	...	...	...	...	...
16.7	...	1	0	1	0	...	...	...	...	...	...	...	...	...	...	...	...
17.2	...	1	2	1	1	...	...	...	...	...	...	...	...	...	...	...	...
17.7	...	...	0	0	2	1	...	1	...	...	...	...	...	...	...	...	...
18.2	...	...	1	0	2	2	...	0	...	...	...	...	...	...	...	...	...
18.7	...	...	3	0	2	2	1	0	...	...	...	...	...	...	...	...	...
19.2	...	...	...	1	1	3	2	1	...	...	...	...	...	...	...	...	...
19.7	...	...	...	1	0	1	2	2	...	...	...	...	...	...	...	...	...
20.2	...	...	...	3	5	2	3	2	1	...	...	...	...	...	...	...	...
20.7	...	...	...	2	2	4	6	3	0	...	...	...	...	...	...	...	...

21.2	...	...	...	1	3	0	6	3	0	...	...	...	...	...	...	...	...
21.7	...	...	...	2	1	1	6	3	2	...	...	...	...	...	...	...	...
22.2	...	...	...	1	4	6	3	1	2	...	...	...	...	...	...	...	...
22.7	...	...	...	...	2	1	2	6	2	...	...	...	...	...	...	...	...
23.2	...	...	...	...	5	6	9	8	4	1	...	...	...	...	...	...	...
23.7	...	...	...	...	2	8	3	7	4	3	...	...	...	...	...	...	...
24.2	...	...	...	...	2	7	9	7	8	1	...	...	...	...	...	...	...
24.7	...	...	...	...	3	10	8	8	7	2	2	...	...	...	...	...	...
25.2	...	...	...	...	2	3	8	5	6	3	2	...	...	...	...	...	...
25.7	...	...	...	...	1	7	7	10	3	4	2	...	...	...	...	...	...
26.2	...	...	...	...	1	3	14	5	4	6	1	2	...	...	...	...	...
26.7	...	...	...	...	...	1	8	7	9	6	3	1	...	...	...	...	...
27.2	...	...	...	...	...	...	5	6	6	2	6	2	...	...	...	...	...
27.7	...	...	...	...	...	...	4	7	4	5	5	0	...	...	...	...	...
28.2	...	...	...	...	...	...	1	8	4	5	5	4	1	...	...	...	...
28.7	...	...	...	...	...	...	...	3	2	3	2	3	0	...	...	...	...
29.2	...	...	...	...	...	...	...	2	4	1	0	0	1	...	...	...	...
29.7	...	...	...	...	...	...	...	4	5	3	2	1	2	...	1	...	...
30.2	...	...	...	...	...	...	...	...	2	2	0	2	0	1	1	...	...
30.7	...	...	...	...	...	...	...	...	1	3	2	2	0	0	0	...	...
31.2	...	...	...	...	...	...	...	...	...	1	0	1	0	0	0	...	...
31.7	...	...	...	...	...	...	...	...	...	1	1	3	3	2	0	...	...
32.2	...	...	...	...	...	...	...	...	...	...	1	2	1	2	1	...	...
32.7	...	...	...	...	...	...	...	...	...	...	0	1	0	2	2	...	...
33.2	...	...	...	...	...	...	...	...	...	...	1	0	2	1	0	1	...
33.7	...	...	...	...	...	...	...	...	...	...	...	0	...	...	1	0	...
34.2	...	...	...	...	...	...	...	...	...	...	...	1	...	...	2	0	...
34.7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	...
35.2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	1	...
35.7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	...
36.2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	1
36.7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...
Totals	12	16	9	13	44	68	107	109	80	52	35	25	10	8	9	3	1
Av. Lengths	10.5	14.8	16.8	20.1	21.4	23.2	24.1	25.3	26.0	27.2	27.9	29.7	31.0	32.0	32.8	35.0	36.2

TABLE III. The numbers of lake trout of given fork lengths in a sample of 311 fish representing age groups 2 to 22 years from Hunter Bay, 1948 to 1958. Measurements as in Table II.

Mid-class length, inches	Age in years																				
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
9.2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
9.7	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
10.2	1	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
10.7	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
11.2	1	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
11.7	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
12.2	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
12.7	...	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
13.2	...	0	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
13.7	...	2	4	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
14.2	...	...	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
14.7	...	...	0	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
15.2	...	...	0	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
15.7	...	...	2	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
16.2	...	...	...	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
16.7	...	...	...	1	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...
17.2	...	...	...	1	2	2	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...
17.7	...	...	...	0	4	4	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...
18.2	...	...	...	1	2	3	0	...	...	...	...	...	...	...	...	...	...	...	...	...	...
18.7	...	...	...	1	3	3	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...
19.2	...	...	...	1	0	2	3	...	...	...	...	...	...	...	...	...	...	...	...	...	...
19.7	...	...	...	0	2	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...
20.2	...	...	...	0	1	0	2	1	...	...	...	...	...	...	...	...	...	...	...	...	...
20.7	...	...	...	1	1	3	1	0	...	...	...	...	...	...	...	...	...	...	...	...	...
21.2	...	...	...	...	1	3	3	2	1	...	...	...	...	...	...	...	...	...	...	...	...
21.7	...	...	...	...	0	1	2	0	1	...	...	...	...	...	...	...	...	...	...	...	...
22.2	...	...	...	...	3	0	3	1	0	...	...	...	...	...	...	...	...	...	...	...	...
22.7	...	...	...	...	3	2	3	2	1	...	...	...	...	...	...	...	...	...	...	...	...
23.2	...	...	...	...	2	1	2	4	3	...	...	...	...	...	...	...	...	...	...	...	...

23.7	...	...	...	...	0	3	1	3	4	...	...	...	...	...	...	...	...	...	...	...	...
24.2	...	...	...	...	0	6	2	2	5	1	...	...	...	...	...	...	...	...	...	...	...
24.7	...	...	...	...	1	4	1	0	5	1	...	...	...	...	...	...	...	...	...	...	...
25.2	...	...	...	...	1	6	6	3	2	4	...	...	...	...	...	...	...	...	...	...	...
25.7	...	...	...	...	...	1	0	1	0	4	...	...	...	...	...	...	...	...	...	...	...
26.2	...	...	...	...	...	...	0	3	3	7	...	...	...	...	...	...	...	...	...	...	...
26.7	...	...	...	...	...	...	3	1	2	4	1	1	...	...	...	...	...	...	...	...	...
27.2	...	...	...	...	...	...	1	1	1	1	2	1	...	...	...	...	...	...	...	...	...
27.7	...	...	...	...	...	...	2	2	1	2	3	3	...	...	...	...	...	...	...	...	...
28.2	...	...	...	...	...	...	0	...	1	1	2	6	...	...	...	...	...	...	...	...	...
28.7	...	...	...	...	...	...	2	...	4	0	1	4	...	...	...	...	...	...	...	...	...
29.2	...	...	...	...	...	...	...	...	1	0	2	4	...	...	...	...	...	...	...	...	...
29.7	...	...	...	...	...	...	...	...	...	1	0	1	1	...	...	...	...	...	...	...	...
30.2	...	...	...	...	...	...	...	...	...	...	0	1	0	...	...	...	...	...	...	...	...
30.7	...	...	...	...	...	...	...	...	...	...	1	2	2	...	...	...	...	...	...	...	...
31.2	...	...	...	...	...	...	...	...	...	...	...	3	2	...	...	...	...	...	...	...	...
31.7	...	...	...	...	...	...	...	...	...	...	...	0	2	1	...	...	...	...	...	...	...
32.2	...	...	...	...	...	...	...	...	...	...	...	0	2	1	...	1	...	...	...	...	...
32.7	...	...	...	...	...	...	...	...	...	...	...	1	1	...	1	0	...	...	...	...	...
33.2	...	...	...	...	...	...	...	...	...	...	...	0	...	...	2	0	...	...	...	...	...
33.7	...	...	...	...	...	...	...	...	...	...	...	1	...	...	1	2	...	...	...	...	...
34.2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	...	...	...	...	...
34.7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2	0	2	...	...	...	...
35.2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	2	...	2	...	...	...
35.7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	2	...	...	...
36.2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	...	2	1	...	...
36.7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	...	...	2	...	...
37.2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	1	1	...
37.7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0	0	...
38.2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	3	...
38.7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...
40.2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
Totals:	2	5	7	11	28	45	41	26	35	26	12	28	10	2	7	7	2	6	5	5	1
Av. lengths	10.7	12.2	14.2	16.6	20.1	21.8	23.1	24.2	25.2	26.2	28.3	29.2	31.4	31.9	33.9	34.7	34.7	35.7	37.0	38.1	40.4

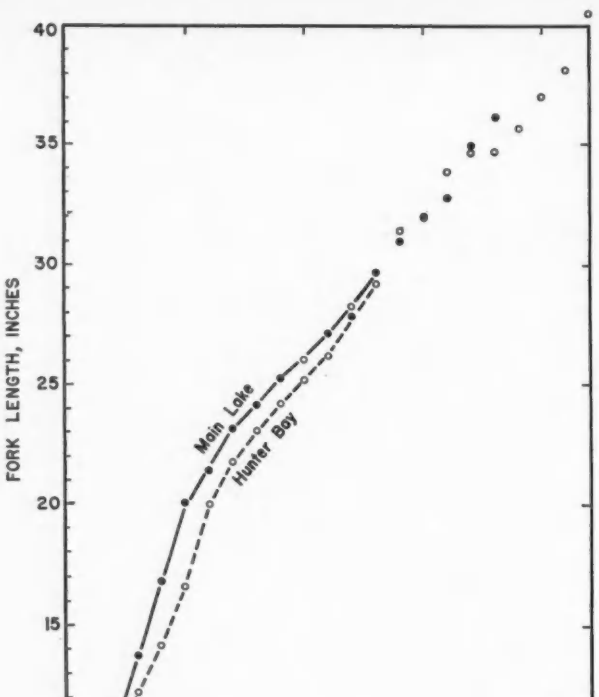

FIG. 3. Average growth in length of lake trout in the main lake and in Hunter Bay, Lac la Ronge.

is the wide variation in growth rate of individual fish. Examination of Table II shows that a fish in its 6th year may range from 13.5 to 26.0 inches, one in its 10th year from 20 to 30.5 inches, and one in its 13th year from 26 to 34 inches. Kennedy (1954) found even greater diversity in the very large samples of trout from Great Slave Lake.

The average rates of growth in trout from the main lake and Hunter Bay are indicated in Fig. 3. The points plotted are the averages shown at the bottom of the columns in Tables II and III. Thus the curve for the main lake is based on a sample of 601 and that for Hunter Bay on 311 trout. It is apparent that trout in the main lake grow fairly rapidly for the first 5 years then begin to slow down. Those in Hunter Bay grow more slowly in the first 4 years then increase and continue steadily to 12 or 13 years at which time they have "caught up" with those in the main lake. Both groups grow at similar rates in the later years as

far as can be determined from the relatively small samples of these very large and old trout. The maximum divergence between the two populations is at 5 years where the average trout in the main lake is 20.1 inches long and weighs 3.5 lb while an average 5-year trout in Hunter Bay is 16.6 inches long and weighs 1.5 lb. It is suggested that young trout in the main lake grow faster because of warmer water and richer food supply but that after the age of 5 years some change in feeding may take place which reverses the advantage and allows the Hunter Bay trout to grow faster and eventually to overtake those of the main lake at the age of about 13 years. An alternative or additional explanation lies in the degree of mixing or exchange of trout between the two areas, suggested by tagging results described below. If substantial mixing occurs, the fast-growing individuals from the main lake might raise the apparent rate in Hunter Bay and slow growing individuals from Hunter Bay might lower the apparent rate of growth of trout in the main lake.

The difference in growth rate between the trout of the main lake and Hunter Bay provides a very interesting parallel with the situation demonstrated by Kennedy (1954) in Great Slave Lake. The western part of Great Slave Lake is shallower, warmer and generally less productive than the eastern part. Trout from the warmer western part grew considerably faster than those from the east (Kennedy's figure 3A, p. 839) especially up to 15 years. From 15 to 19 years the rate of growth of trout in the eastern part of the lake was considerably faster than that in the west.

Various authors have compared the rate of growth of lake trout in different areas, referring to faster growth near the southern limits of its distribution and slower in the far north. Very rapid growth has been reported in the Finger Lakes of New York State by Royce (MS, 1943) and in the Waterton Lakes in south-western Alberta by Cuerrier and Schultz (1957). Fast growth was also found in Lake Opeongo and other small lakes of Algonquin Park, Ontario, by Fry and Kennedy (1937) and Martin (1952). Intermediate to slow growth has been found in large lakes of northern Saskatchewan (Rawson, 1959) and in Great Slave Lake (Kennedy, 1954). The slowest growth reported for lake trout appears to be that in Great Bear Lake (Miller and Kennedy, 1948). The rate of growth of trout in Lac la Ronge, as indicated in Fig. 3, is very close to that in Cree and Wollaston Lakes (Rawson, 1959) and intermediate between the rates shown by Kennedy (1954) for the east and west part of Great Slave Lake. It is also similar to that recorded for trout in Lake Michigan by Cable (1956).

The relation of length to weight of the Lac la Ronge trout was determined by plotting the lengths and weights of most of the specimens recorded in Tables II and III and drawing the best curve. The result was verified by averaging the weights of all individuals falling in certain length classes. No significant differences were found in the length–weight relationship of males and females nor between trout from the main lake and Hunter Bay. The results are presented in Table IV. While the young trout are about the same weight as those of similar lengths in Great Slave (Kennedy, 1954, table 1) the old trout (over 15 years) in Lac la Ronge become 25 to 50% heavier.

TABLE IV. Average fork length and average weight in pounds of lake trout in Lac la Ronge.

Inches	Pounds	Inches	Pounds	Inches	Pounds	Inches	Pounds
7	0.10	16	1.41	25	7.2	34	20.5
8	0.16	17	1.78	26	8.2	35	22.5
9	0.28	18	2.1	27	9.2	36	25.0
10	0.41	19	2.6	28	10.4	37	27.0
11	0.55	20	3.4	29	11.8	38	30.0
12	0.68	21	4.1	30	13.2	39	32.0
13	0.84	22	4.8	31	14.9	40	35.0
14	1.02	23	5.6	32	16.8	41	38.0
15	1.21	24	6.3	33	18.8	42	40.5

Only a small percentage of the trout in Lac la Ronge survive past their 19th year, at which time the average length is about 36 inches (91 cm) and the average weight about 25 lb (11.2 kg). The largest for which we have a scale sample was 40.5 inches (101 cm) in length, weighed 34.5 lb (15.6 kg) and its age was interpreted as 23 years. Lake trout weighing 30 lb or more are naturally of tremendous interest to the angler, and although they represent only a small fraction of the population they are by no means rare. Some of the lengths and weights of large trout taken in Lac la Ronge during the last 10 years are recorded in Table V. Only those measured and weighed by fisheries research personnel or officers of the Department of Natural Resources are included. More than

TABLE V. Fork lengths and weights of some very large trout caught in Lac la Ronge.

	Inches	Pounds		Inches	Pounds
1949	38.0	37.2	1956 (cont'd)	38.5	32.5
	38.5	32.5		40.0	37.3
1950	37.7	30.5	1957	38.0	32.0
	38.5	34.5		42.0	33.0
	40.5	32.0	1958	39.0	35.0
1951	40.0	34.0		41.0	32.0
	40.5	34.5	1959	38.0	33.1
1952	41.0	37.0		38.5	36.0
1953	39.5	36.2		40.5	33.7
	40.5	34.5		42.2	37.5
	42.0	41.0		47.0	37.0
1954	39.5	32.5		48.1	43.0
1955	40.5	28.5	1960	34.5	30.5
	41.0	28.5		38.5	32.5
1956	36.5	36.2		40.5	38.5
	38.0	32.5		42.0	34.0
				46.0	41.5

half of these very large trout were caught in Hunter Bay or in the Narrows which joins Hunter Bay with the main part of Lac la Ronge. In 9 of the 11 years represented in Table V, a trout from Lac la Ronge was the largest caught in Saskatchewan.

FOOD

The stomach contents of all trout taken in sampling were examined and recorded in the field but any material not readily identified was preserved for laboratory analysis. Of 511 stomachs examined 257 were empty. The younger fish took somewhat different food than the older ones, so the two groups have been considered separately. The first is made up of those with fork lengths up to 16 inches (40.6 cm), which includes all of the 2-year trout, most of the 3-year-olds and a few 4-year-olds.

Forty-two trout from 7 to 16 inches (17.7–40.6 cm) fork length were obtained in the course of the investigation. Nineteen of these were empty and the stomach contents of the remaining 23 are recorded in Table VI. The main invertebrate food was *Mysis relicta*, followed by moderate numbers, but little volume, of *Pontoporeia affinis* and Entomostraca. The fish eaten included mainly

TABLE VI. The food of 23 small trout 7–16 inches (18–41 cm) in fork length in Lac la Ronge; the stomachs of 19 additional trout were empty.

	Percentage frequency of food item in stomachs	Estimated percentage of total volume of food
Mysis	25	17
Pontoporeia	14	2
Entomostraca	9	+
Chironomid larvae and pupae	14	+
Ciscoes	9	31
Sculpins	17	21
Ninespined sticklebacks	26	18
Unidentified fish	16	9

ciscoes, *Leucichthys zenithicus* and L. *tullibee*, ninespine sticklebacks, *Pungitius pungitius*, and sculpins. Of 17 sculpins taken from the trout stomachs 9 were deepwater sculpins, *Triglopsis thompsoni*, and one was *Cottus cognatus*. *Mysis* was particularly numerous in the stomachs of 2-year-old fish (7 to 12 inches) and is undoubtedly a very important food of young trout in Lac la Ronge, as it is in Lake Superior (Eschmeyer, 1956), Great Slave Lake (Rawson, 1951), Keuka Lake, New York (Royce, 1951) and elsewhere. The deepwater sculpin is apparently one of the first fish of suitable size and in suitable location to provide food for young trout. The frequent feeding on ninespine sticklebacks suggest that this species is also common in the deep water. Ciscoes, although a main food of the larger trout, were found in only two trout under 16 inches.

The food of larger trout, 16 to 40 inches (40.6–101) fork length, is summarized in Table VII. This represents the examination of the 469 stomachs, 231 containing food. The fish items make up 90% of the food occurrences and invertebrates barely 10%. The fish eaten include 10 of the 19 species recorded for the lake (Rawson and Atton, 1953) but only the first 6 listed in Table VII

TABLE VII. Occurrence of food items in stomachs of 231 large lake trout from Lac la Ronge; 238 additional stomachs were empty.

Food items	Number of stomachs	Percentage frequency
Unidentified fish remains	132	57.1
Ciscoes, *Leucichthys* spp.	53	22.9
Whitefish, *Coregonus clupeaformis*	13	5.6
Ninespine stickleback, *Pungitius pungitius*	13	5.6
Longnose sucker, *Catostomus catostomus*	5	2.2
Yellow perch, *Perca flavescens*	5	2.2
Sculpins, *Triglopsis* and *Cottus*	5	2.2
Burbot, *Lota maculosa*	2	0.87
Walleye, *Stizostedion vitreum*	2	0.87
Spottail minnow, *Notropis hudsonius*	2	0.87
Lake trout, *Cristivomer namaycush*	1	0.43
Mysis relicta	16	6.93
Pontoporeia affinis	3	1.29
Mayfly nymphs	2	0.87
Stonefly nymphs	1	0.43
Winged ants	2	0.87

appear in significant quantities. The ciscoes are clearly the main food of adult trout in Lac la Ronge. *Mysis* is the only invertebrate food of common occurrence, The volumes of the individual food items in trout stomachs were not measured. However, considering the relative size of the food organisms and the average numbers found in individual trout stomachs, it is possible to make a rough estimate of the contribution of different items. Ciscoes probably provide about 50% of the food of large trout, whitefish about 15% and all other fish about 30%. The invertebrates, although of frequent occurrence, are small in size and probably provide less than 5% by volume of food of these trout.

In two other lakes of northern Saskatchewan, Cree and Wollaston (Rawson, 1959), fish provide 95% and 85% of trout food by volume, with ciscoes the most important species followed by whitefish, suckers and other species. In Great Slave Lake ciscoes were also the major trout food, followed by cottids, burbot, whitefish and longnose suckers, and with *Mysis* again the main invertebrate food (Rawson, 1951). Trout in Lake Athabaska had a similar diet (Rawson, 1947). In Lake Nipigon, Ontario, Clemens *et al.* (1924) report ciscoes as the main food followed by sticklebacks, cottids, suckers and whitefish. McCrimmon (1956) records the cisco as the main food of trout in Lake Simcoe, Ontario. It is clear that in most of the large lakes in Canada, the larger lake trout feed mainly on fish and especially on ciscoes. In small lakes, such as those of the Algonquin

Park, Ontario, plankton and surface and bottom insect foods are taken in large amounts (Ricker, 1932; Martin, 1952). In small lakes of the Northwest Territories the lake trout had eaten mostly fish but insects were found in nearly half the stomachs and molluscs and crustaceans in a few (G. Hunter, Fisheries Research Board of Canada, Arctic Unit, personal communication).

In the deeper water of Lac la Ronge where the lake trout is the dominant piscivore, the burbot is probably its main competitor, eating considerable quantities of ciscoes. In the main lake where, judging from our standard net sets the burbot is more abundant than the lake trout, this competition may have an important effect on trout production. In Hunter Bay, where trout appear to outnumber burbot by about 3 to 1, the competition would have less significance. Pike and walleyes also feed extensively on several of the fish species which are eaten by the trout. This competition would be most active in the spring and fall when the trout come into shallow water.

PARASITES

Internal parasites were collected from the lake trout and additional specimens were recovered from the preserved stomach contents. We are indebted to Dr Betty-June Myers, Institute of Parasitology, Macdonald College, for identification of these materials.

Cestodes collected from the alimentary canal were mostly *Eubothrium* (probably *salvelini*) with smaller numbers of *Proteocephalus* sp. A few cysts of the cestode *Triaenophorus crassus* were found in the flesh. This parasite is widespread in the trout of northern Saskatchewan and the Northwest Territories but apparently not numerous except in a few lakes, mostly of small size.

Unidentified nematodes, mostly larval, were found in the intestine and occasionally in the swim bladders of the trout. Nematodes in the swim bladders of 4 lake trout from Little Bear Lake, 40 miles south of Lac la Ronge, were identified as *Cystidicola stigmatura* by Dr R. C. Anderson, Ontario Research Foundation, Toronto.

An external copepod parasite, *Salmincola siscowet*, was frequently found attached to the body surface, usually on the fins. We are indebted to Dr Paul Illg, University of Washington, for this identification.

THE DISTRIBUTION OF TROUT IN LAC LA RONGE

It is well known that the distribution of trout in the lake is by no means uniform at any season nor the same in different seasons. In a general way trout movements are understood to be related to seasonal changes in temperature and to such vital activities as feeding and spawning. It is of special biological interest to determine the effects of temperature more exactly and to follow the seasonal movements of the trout population. Such information is of course vital to the success of the angler, the commercial fisherman and to the administrator concerned with utilization and protection of fish stocks.

The primary source of information concerning trout distribution has been systematic gill netting throughout several summers and in all parts of the lake. Depths, temperatures and feeding activities were recorded for each set. Tagging and recovery have provided important information on the movements of individual trout. Spawn-taking operations in 5 years have made it possible to examine large samples of mature trout and to tag many of them. Forty years of records of commercial fishing, both summer and winter, and interviews with experienced fishermen have helped to complete the picture, especially concerning winter distribution.

Seasonal Distribution in Relation to Depth and Temperature

Gill nets used in sampling the lake trout population were in the form of standard gangs 300 yards (275 m) in length and made up from six 50-yard lengths of 1½-, 2-, 3-, 4- and 5½-inch stretched mesh. These were set along the lake bottom for overnight periods averaging about 22 hours. Uniform specifications and techniques of setting were maintained throughout the 10-year period, with one important exception. In the first 4 years, from 1948 to 1952, cotton and linen nets were used but from 1953 to 1959 nylon nets were always used. In 1958 and 1959 the netting program was planned specifically for the trout investigation. Thus care was taken that in every 2-week period nets were set in all depth ranges and in different areas of the lake. The catch from each mesh size was separated and depths were measured at each end and in the centre of the net so the depth at which each trout was caught could be recorded. At each set a vertical series of temperatures was taken at 1-metre intervals with an electrical resistance thermometer.

The depth distribution of trout in Lac la Ronge in the summers of 1958 and 1959 is presented in Fig. 4. Isotherms in degrees Centigrade at 2-degree intervals have been plotted for the periods May to September. Lac la Ronge is a very large lake with a northern islands region and a southern open area. Thermal stratification is relatively stable in deep basins between the islands at the north and rather unstable in the exposed southern area. (Atton, MS, 1955b). Thus a set of isotherms for the whole lake can indicate only the generalized condition for the whole area. Temperature series at individual stations during late July and August, 1958, showed sharp thermoclines with as much as 7°C drop in temperature in 5 m. Autumn circulation usually occurs first in the open water (about Sept. 9 in 1952), a little later in the islands area (Sept. 12, 1952) and still later in Hunter Bay. Superimposed on the isotherms are vertical polygons representing the total numbers of trout caught in each 5-metre depth unit over a half-month period. These polygons represent the sum of the catch in the half-month period from the number of sets made in this period. Thus they do not represent equal fishing efforts.

The upper chart in Fig. 4 represents the season of 1958. The breakup of ice was completed on May 11 and observations began on May 20. In the remainder of May trout were caught in all depths from 5 to 35 m with the greatest number

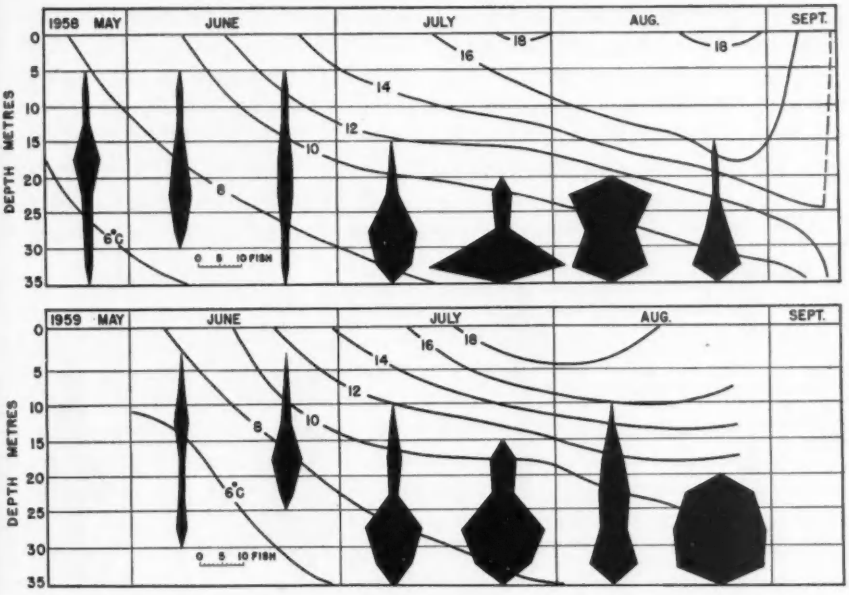

FIG. 4. Charts showing the vertical distribution of lake trout caught by gill nets in relation to the water temperatures through the summers of 1958 and 1959, Lac la Ronge.

between 15 and 20 m. Surface temperatures increased from 6.7 to 9.6 and bottom from 5.5 to 6.2°C. In the two halves of June, trout continued to be caught in almost all depths and with no great concentration at any level. However, by July 1 the surface water had warmed beyond 14° and the 10° isotherm had dropped below 15 m. In the first two weeks of July no trout were caught above the 15-metre level and the maximum catch was between 25 and 30 m where temperatures were mostly between 8 and 9°C. In the second half of July and throughout August, as the thermal stratification became more pronounced, very few trout were caught above 20 m. Thermal stratification was destroyed in early September in the open lake and a little later among the islands. Although no test netting was possible at this time in 1958, it is known that trout came up into shallow water where they were taken in large numbers by anglers casting at the surface.

In 1959 the ice remained on Lac la Ronge until May 27 and thermal events were nearly 2 weeks behind those of 1958, throughout the summer (Fig. 4). Lake trout were caught fairly uniformly at most depths throughout June. In the first half of July the 10° isotherm had gone down to 15 m and the trout were beginning to concentrate in the deep water, 25 to 35 m. In the last half of July this deep concentration was still greater. Because of the late season the depth of heating in 1959 was not as great as that in 1958 and the 10° isotherm barely reached the 25-metre level. However, the surface water was warmer in 1959

and thus the "thermocline" was more sharply defined than in 1958 (a range of 6° in about 14 m in August, 1958, and 6° in 10 m in August, 1959).

Hunter Bay, about 30 miles from laboratory headquarters, was sampled less often than the main lake. However, in 1958 sufficient data to demonstrate the vertical distribution of trout with temperature were obtained for one-half of June, July, August and September (Fig. 5). In the first half of June, with

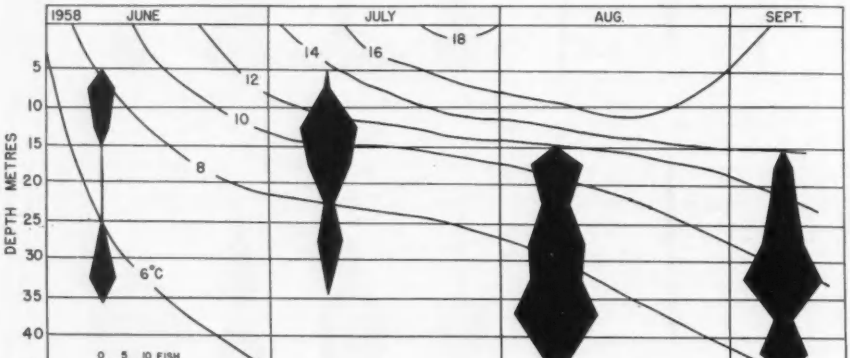

FIG. 5. Chart showing the vertical distribution of lake trout in relation to water temperatures in Hunter Bay, Lac la Ronge, 1958.

bottom temperatures about 6° and surface 8.5°, trout were caught in about equal numbers in shallow (5–15 m) and deep (25–35 m) waters. In early July, while thermal stratification was beginning, considerable numbers of trout were still caught between 10 and 30 m. In the first half of August no trout were caught above 15 m but considerable numbers at all depths from 15 to 45 m. A similar situation was observed in the first week of September. As in the main lake 1948 and 1949 (Fig. 4) trout were mostly caught below the 10° isotherm—a few between 10 and 12° and very few between 12° and 14°.

Having measured the temperature at net level for every gill net in 1958 and 1959 it was possible to tabulate the distribution of the trout caught by temperature as follows:

Temperature class, °C[2]	5	6	7	8	9	10	11	12	13	14
No. of trout caught	5	39	29	171	147	111	3	3	0	1

Thus of 519 trout caught in the two seasons only 1.35% were from water warmer than 11°C and 22.7% from water warmer than 10°C. It can be inferred that the trout in Lac la Ronge prefer temperatures of less than 10°C (50°F). Ferguson (1958) in an extensive study of preferred temperatures of freshwater fish records 12°C as the upper limit of preferred temperature for the lake trout.

[2]Temperatures were read to the nearest tenth-degree; the class 5 includes readings from 5.0° to 5.9°, etc.

In order to remain in water cooler than 10° or 12°C the trout move down in July and August to depths of at least 15 and usually 20 m (Fig. 1). In Hunter Bay where nearly one-half its area is deeper than 20 m this will not necessitate much horizontal movement. In the main lake, however, about 85% of the water area is less than 20 m deep so the trout, to remain in cool water, must move into the restricted deeper areas. The resulting concentration of trout in the deep "holes" is most fortunate for the anglers who continue to catch trout effectively in these regions during July and August.

MOVEMENTS OF TROUT AS INDICATED BY TAGGING AND RECOVERY

Lake trout were first tagged in Lac la Ronge in 1950. A few were tagged in 1952 and larger numbers in 1958 and 1959. Specimens for tagging were selected from the gill-net catch, taking only those which were active and showed no signs of damage from the nets. In 1950, 1952 and 1958 Petersen type tags with half-inch diameter plastic discs were used. These were attached with monel metal pins just below the dorsal fin. In 1959 a button-type tag was used attached to the operculum by means of special pliers as described by Cable (1950). Tags were returned from fish caught by anglers and from gill nets set by domestic fishermen, our own standard gangs and especially from those used by hatchery personnel to obtain trout for spawn taking.

Returns from 429 trout bearing Petersen tags numbered 60, or 14%. From 322 marked with button-type tags only 1 was recovered. The reason for this discrepancy is not known.

A summary of the trout tagged with Petersen tags and the recoveries to December, 1960, is presented in Table VIII. Those tagged throughout the summers of 1952, 1958 and 1959 were taken in our experimental nets in all parts of the lake. Those at the spawn camp were tagged in late September or early October at a point about 2 miles south of Bear Island, Fig. 6.

The maximum distance from the point of tagging to the place of recovery was 22 miles. Nine trout were recovered from 15 to 22 miles from the point of tagging, 12 at distances 4 to 14 miles and 8 at distances of 1 to 4 miles. The average distance for these 29 trout was 8.8 miles. Another 31 trout, tagged at the spawn camp, were recovered within a mile of the point of tagging in the years which followed.

The time from tagging to recovery of lake trout in Lac la Ronge has varied from one month to nearly 3 years. Approximately 12% were recaptured in the calendar year in which they were tagged, 60% in the second year and 28% in the third year.

The locations of tagging and recapture of 24 trout which were caught at some distance from the site of tagging, are indicated in the sketch map, Fig. 6. Thirty-six other trout, which had travelled only short distances, have not been

TABLE VIII. Recovery of lake trout marked with Petersen type tags in Lac la Ronge, 1950, 1952 and 1958. (Of 123 tagged during summers, 15.4% were recovered; of 306 tagged at spawning, 13.4% were recovered.)

	1950			1952	1958			All years
	Summer	Spawning	Total	Summer	Summer	Spawning	Total	
Number of trout tagged	56	131	187	28	67	147	214	429
Recoveries, angling								
Same year	2	0	...	0	2	0	...	
Next year	2	2	...	4	2	3	...	
Third year	0	2	...	0	2	2	...	
All years	4	4	8	4	6	5	11	23
Recoveries, domestic and experimental nets								
Same year	2	0	...	0	0	0	...	
Next year	1	2	...	0	1	0	...	
Third year	0	0	...	0	3	1	...	
All years	3	2	5	0	4	1	5	10
Recoveries, nets at spawning								
Same year	1	0	...	0	0	0	...	
Next year	0	0	...	0	1	18	...	
Third year	0	5	...	0	0	2	...	
All years	1	5	6	0	1	20	21	27
Total recoveries	...	...	19	4	...	...	37	60
% recovered	...	...	10.2	14.3	...	...	17.3	14.0

plotted. Trout tagged in the spawn camp area south of Bear Island have been recaptured in such remote areas as Pickerel Bay (in the southeast part of Hunter Bay), Fox Point on the south shore, south of the La Ronge townsite, off the inlet of the Nemeiben River, and in Four Portages and Pipestone Bay at the north. A trout tagged near Hunter Narrows has crossed the lake to Nut Point on the west and one tagged south of Nut Point went northeast into Four Portages Bay. Of particular interest is the interchange of trout between the main lake and Hunter Bay. Two trout tagged in Hunter Bay were recaptured some 15 miles west at Big Island and Bear Island respectively. Three trout tagged in the main lake were recaptured in Hunter Bay. Thus 5 of a total of 60 recaptures have passed through Hunter Narrows suggesting that a moderate rate of exchange occurs between the two populations. If this rate applies to all trout randomly and continues over a period of 5 to 10 years, the resulting mixture of the two populations may account for part of the apparent convergence of growth rates shown for the two areas in Fig. 3.

In the years 1950 and 1958, 278 trout were tagged on the spawning grounds. From this group 25 have been recovered in spawning runs 1 and 2 years later

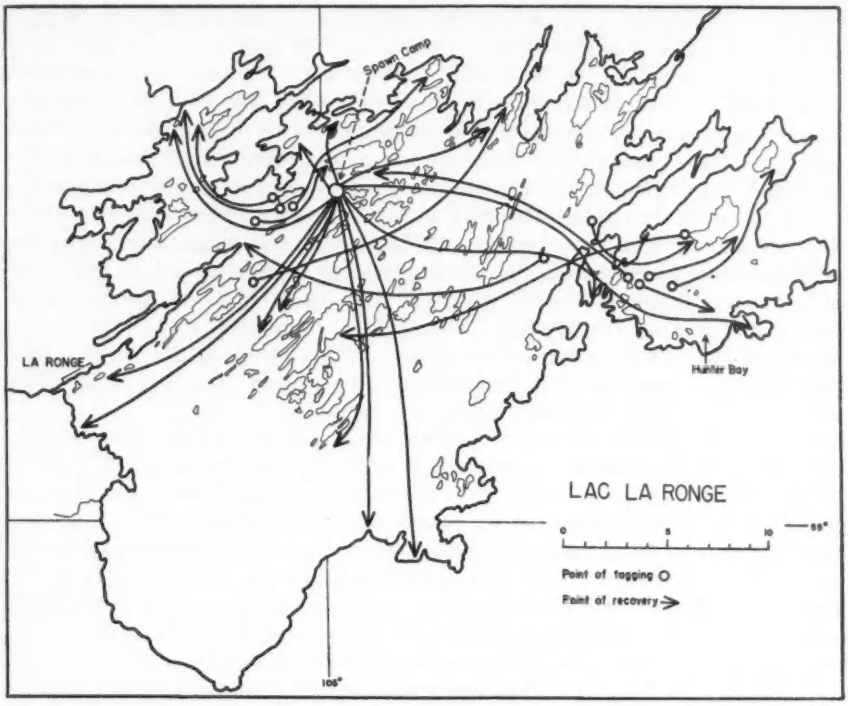

Fig. 6. Location of tagging and recovery for 24 lake trout in Lac la Ronge. Note that 30 other trout were recaptured a short distance from their place of tagging.

at the same location, and 12 have been recovered elsewhere in the lake at various seasons. This result suggests a considerable degree of "homing" of trout to the same spawning grounds. Unfortunately, it has not been possible to carry on autumn sampling on other spawning grounds to test the degree of mixing of different runs. Homing of lake trout has been demonstrated by Martin (1960) in small lakes of the Algonquin Park, but McCrimmon (1958) found no evidence of homing in tagging studies in Lake Simcoe, Ontario.

Other Information Concerning the Distribution of Trout

The spawning run has been referred to above in the discussion of life history. Since there are many known spawning grounds in different parts of the lake it is unlikely that trout from any area would need to move more than 2 or 3 miles to spawn. In terms of population movements, it should be noted that the spawning migration would not affect the large group of trout less than about 23 inches length and 7 years of age. It is probable also that about two-thirds of the mature

trout in Lac la Ronge spawn in any one year, thus one-third of the mature trout might not migrate at spawning time.

Careful analysis of the food taken by lake trout and their association with other species of fish in the gill nets failed to provide any evidence of daily or seasonal migrations of trout for feeding purposes. Since the ciscoes are the main food of trout the association of these two species was examined. Trout were very frequently caught in the small mesh nets when attempting to eat small ciscoes already gilled in the twine. However, in net catches where ciscoes were particularly abundant (more than 100 per set) lake trout were usually few and often were absent. It would seem that the various fish on which the lake trout feed in Lac la Ronge are sufficiently abundant that the trout do not need to make any special feeding migrations. This may be contrasted with lakes in the Algonquin Park, Ontario, where Fry (1939) and Martin (1952) have shown that lake trout may come up through the thermocline to feed on yellow perch which are concentrated in the warmer water.

The field program for this study did not include netting for trout beneath the ice. However, extensive commercial netting in the winters from 1922 to 1945 took an average of more than 100,000 lb of trout per year. Careful questioning and comparison of statements from several commercial fishermen who took part in these operations have provided useful information concerning winter distribution of the lake trout. The winter fishing began on December 1 about 10 days after the ice covered the lake. Gill nets were usually $5\frac{1}{2}$ inch stretched mesh, 36 meshes high and about 17 feet (5.2 m) in height. These were set at first in 30 to 60 feet (9 to 18 m), sometimes fairly close to shore. In January and February the trout tended to move offshore and somewhat deeper but nets were rarely set in more than 80 feet (24 m) of water. Trout could be caught in all parts of the lake, but about five main fishing areas were used. The western part of the open water was fished from Birch Island 3 miles south of Potato Point. A northwestern area was fished from camps on Nut Point, Freeman Island and Moose Point. The centre island group had camps on Brooks and Big Islands and south to Meraste Point. A fourth major fishing ground was in Hunter Narrows and Trout Narrows, 3 miles to the south. The fifth main camp was in Hunter Bay on Rainy Island. With the exception of the Birch Island region these are all still considered trout grounds. It is of interest that very heavy trout catches were made in this area of moderate depth near Birch Island in 1921 and 1922 and again for several years following 1940.

In summary, movements in response to water temperature are the main factor in explaining the seasonal distribution of trout in Lac la Ronge. The spawning migration brings most of the larger fish into shallow water at the beginning of October. Tagging has demonstrated rather extensive wandering of perhaps one-third of the population and exchange between the main lake and Hunter Bay of perhaps one-tenth. When the ice breaks many trout come into shallow water and remain there until warming forces them to move down. In July when thermal stratification is well established, the trout are at least 15 m down and in August, below 20 m (Fig. 1). They thus remain in water cooler than

12° and usually below 10°C. In September, when the surface water cools and autumn circulation occurs, the trout again come into shallow water. Even after the spawning period, trout can be caught in shallow water. Evidence from commercial fishing indicates that they are in moderate depths in early winter and tend to be widely dispersed during later months of ice cover.

POPULATION DATA

Information as to the abundance of lake trout in Lac la Ronge has been obtained by extensive use of standard gangs of gill net and less directly from the creel census and records of commercial catch. The catches in standard gangs provide some measure of the availability of trout more than 2 or 3 years of age. They also provide materials from which size distribution, year-class composition and rate of growth has been determined. Our limited program of tagging and recovery was designed simply to follow movements of fish and the results are not considered suitable for making estimates of the population of trout in the lake.

A summary of gill net catches in Lac la Ronge is presented in Table IX. These include 169 catches made in 1948, 1949, 1958 and 1959, in the main lake and in Hunter Bay. Hunter Bay is separated because it differs from the main lake in depth, temperatures, trophic condition and fish population. Gill netting in the period 1950 to 1957 was concentrated in certain areas and depths for the study of whitefish, walleyes (pickerel) and for other purposes. Thus the catch data for these years were not comparable to those in Table IX and are not included.

The general composition of the fish population in Lac la Ronge is suggested by the data in Table IX. Whitefish usually make up 40 to 50% of the catch numerically, ciscoes 30 to 40%. Two species of suckers contribute about 10%

TABLE IX. Summary of average numerical composition and total weight of gill-net catches in Lac la Ronge proper and Hunter Bay 1948, 1949, 1958 and 1959.

	Main lake						Hunter Bay			
Number of gill-net sets	1948–49 53		1958 41		1959 38		1948–49 15		1958 22	
	no.	%	*no.*	%	*no.*	%	*no.*	%	*no.*	%
Whitefish	64.2	40.3	72.2	39.2	79.7	42.6	57.7	60.6	59.1	53.2
Cisco	67.8	42.5	77.5	42.1	68.7	36.6	22.1	23.2	32.6	29.5
Longnose sucker	11.5	7.2	18.3	9.9	22.9	12.2	1.5	1.6	5.5	5.0
White sucker	5.1	3.2	0.56	0.38	1.4	0.76	0.9	1.0	2.5	2.2
Lake trout	1.9	1.2	3.9	2.0	4.9	2.6	4.6	4.8	7.9	7.1
Pike	2.2	1.4	0.88	0.47	0.81	0.44	2.3	2.4	0	0
Walleye (pickerel)	1.6	1.0	1.0	0.52	0.81	0.44	4.7	4.9	1.0	0.9
Burbot	2.0	1.2	9.0	4.9	7.8	4.3	1.4	1.5	1.6	1.48
Perch	3.2	2.0	1.2	0.6	0.31	0.17	0	0	0	0
All species number	159.5	...	184.5	...	187.3	...	95.2	...	110.2	...
Approx. av. weight per set pounds	152.	...	177.	...	180.	...	126.	...	151.	...
Kilograms	69.0		80.2		81.6		57.1		68.5	

in the main lake but much less in Hunter Bay. The lake trout follows these with about 2% by numbers in the main lake and 5% in Hunter Bay. Because of their large average size the lake trout represent nearly 12% by weight of the catch in the main lake and about 26% by weight of the catch in Hunter Bay.

Since the lake trout depends mainly on plankton-feeding and bottom-feeding fish for its food, the ratio of trout and other piscivores to non-piscivores is of considerable interest. The numerical data bearing on this question have been extracted from Table IX and pertinent information regarding weights added to form Table X. The four plankton- and bottom-feeding species (whitefish,

TABLE X. Percentage of piscivores compared with that of plankton and bottom feeding fish, numerically and by weight in the gill-net catch in the main lake and Hunter Bay, Lac la Ronge.

	Main lake % of catch		Hunter Bay % of catch	
	Numerically	By weight	Numerically	By weight
Four plankton and bottom feeders (Whitefish, ciscoes and two suckers)	92	75	88	64
Five piscivores (Trout, pike, walleye, burbot and perch)	8	25	12	36
Trout alone	2.9	11.5	5.9	26

ciscoes and two suckers) outnumber the piscivores, trout, pike, walleye and perch by 92% to 8% in the catch from the main lake and 88% to 12% in Hunter Bay. The comparable proportions by weight are 75 to 25 in the main lake and 64 to 36 in Hunter Bay. Thus in the catch from the main lake non-piscivores outweigh piscivores by 3 to 1 and in Hunter Bay by 1.8 to 1. Since all species of fish are not equally vulnerable to gill nets set along the bottom of a lake it should not be assumed that the proportions of various species in the catch as recorded above represent the true proportions of these species in the total population. Extensive use of gill nets set vertically in Lac la Ronge in 1959 indicated that the ciscoes are often much more available at levels far above the bottom. Thus the ciscoes probably represent a much larger fraction of the total population than would be suggested by the values in Table IX. An important difference between the two parts of the lake is that in the catch from Hunter Bay the lake trout make up two-thirds of the number of piscivores but in the main lake only one-quarter. This is consistent with our knowledge that Hunter Bay is essentially a deep, cold, oligotrophic lake ideally suited to lake trout whereas the main lake is more mesotrophic with much shallow water, better adapted to burbot, pike, and walleye. In the catch from the main lake the burbot outnumbers the lake trout and is no doubt its main competitor for food. In the Hunter Bay catch the lake trout greatly outnumbers the burbot so the latter is probably of minor significance as a competitor in this region.

The availability of lake trout to gill nets in Lac la Ronge may be compared to that in five other lakes in northern Saskatchewan which have been tested with similar standard gangs of net (Rawson, 1960). The numerical percentage of trout in the average catch from these lakes is as follows: Reindeer Lake 16.8; Wollaston, 11.2; Hunter Bay, 4.8[3]; Cree, 4.5; Athabaska, 2.4; La Ronge, 2.2[3]. A small amount of trout was caught in Amisk and Ile à la Crosse but no percentage recorded. It will be seen that Hunter Bay ranks third and the main part of Lac la Ronge sixth in the gill net catches of trout in these large lakes of northern Saskatchewan.

The average numbers of fish of all species caught in standard gang sets in 1958 and 1959 are greater than those caught in 1948 and 1949 both for the main lake and Hunter Bay, Table IX. The increase in the main lake and in Hunter Bay is about 16%. This increase is somewhat less than that which might be expected from the greater efficiency of nylon mesh nets.

The increase in catch of lake trout after 10 years was greater than that for most other species. In the main lake the average catch increased from 1.9 to 4.4 and in Hunter Bay from 4.6 to 7.9 trout per set. Specific data on the effectiveness of nylon and cotton nets in capturing lake trout in Lac la Ronge was obtained by Atton (1955a). He found that for nets of 1.5-, 2- and 5-inch stretched mesh the ratio of trout caught in cotton to nylon net was 1:1.26. The ratios of trout caught in 1948 to 1949 to those caught in 1958-59 are 1:2.3 in the main lake and 1:1.7 in Hunter Bay. Since the increase in catch after the 10-year period is more than twice as great as that which would be expected from the change to nylon nets, it seems safe to conclude that the availability of trout in Lac la Ronge has increased in the 10-year period. It will be noted later that the anglers' catch of trout has maintained a high level since 1952 and has increased in the years 1958 and 1959.

Changes in a fish population resulting from exploitation are commonly reflected in a decrease in the average length of fish taken in standard gear. The length distribution of trout taken in standard gangs from 1948 to 1959 is indicated in Fig. 7. A group of 325 trout caught from 1948 to 1953 had an average fork length of 23.08 inches and a prominent mode at 24 inches. In 1958, our sample of 304 trout averaged 23.15 and in 1959, 168 trout averaged 23.77 inches. Thus there is no evidence of change in the average length of trout in Lac la Ronge during this period.

An analysis of the age–length data was made to see whether any change in the year-class composition had taken place. Table XI compares the two groups. In both, the 8-year class is the most numerous and other year classes from 6 to 12 are represented by substantial numbers. Again no change is evident over the 10-year period.

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[3]These percentages differ somewhat from those in the earlier paper (Rawson, 1960) because they include extensive data from 1958 and 1959. The sequence among the seven lakes remains the same.

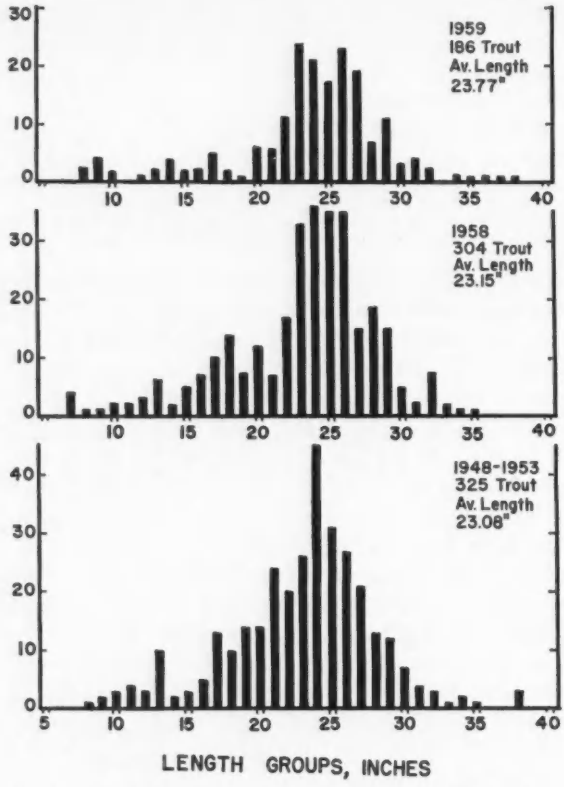

Fig. 7. Length frequencies of three samples of lake trout taken in standard gangs of gill net, Lac la Ronge, 1948 to 1959.

TABLE XI. Percentage composition by age-groups of trout taken in standard-gang nets in 1948–49 and in 1958.

	Number of trout	Age-groups								
		3–5	6	7	8	9	10	11	12	13–16
1948–49	118	7.4	8.5	15.4	22.8	17.6	11.4	7.4	4.0	5.5
1958	160	8.1	11.5	13.4	19.4	16.0	11.2	7.4	4.1	8.9

UTILIZATION OF THE TROUT POPULATION

The history of fishing in Lac la Ronge falls naturally into three periods. The first was a time of domestic fishing, when Indians, traders and travellers made use of the abundant fish population. Trading posts existed on the lake nearly 200 years ago and the sites of at least four are known. Records of the amounts of fish taken are very scanty but it is reasonable to assume that the fish taken

to feed the small number of residents and their dogs would have had little effect on the fish population.

The period of commercial fishing began about 1920 and the first records of production indicate a catch in 1922 of 229,500 lb, including 72,200 lb of trout. Taken by gill netting through the ice, these fish were frozen and hauled by horses 180 miles to rail-head at Prince Albert. After about 1930 most of the winter hauling was done by tractor. In 1945 a fish processing and freezing plant was built which allowed summer as well as winter fishing in 1946 and subsequent years. The commercial production of this period of nearly 30 years is shown in Table XII. The average annual trout production through this period was 113,505 lb.

TABLE XII. Commercial fish catch, in pounds dressed weight (head on) from Lac la Ronge in the years 1922–1950. From Rawson and Atton (1953).

Fiscal year ending March 31	Whitefish	Trout	Pickerel	Pike	Suckers	Others	Total
1922	69,300	72,200	20,900	27,700	21,700	17,700	229,500
1923	23,700	78,800	2,800	10,400	14,500	12,700	142,900
1924	53,200	91,300	6,800	8,100	10,700	7,200	177,300
1925	100,800	149,200	22,600	26,200	30,700	30,800	360,300
1926	214,200	188,000	23,000	23,600	37,200	37,000	523,000
1927	284,100	176,900	17,300	30,700	84,700	100,300	694,000
1928	336,900	184,900	22,900	20,100	27,800	25,800	618,400
1929	348,600	182,900	24,300	22,000	38,700	30,200	646,700
1930	251,900	164,600	14,300	18,100	26,500	22,500	497,900
1931	374,000	191,628	7,600	30,600	33,200	10,400	647,428
1932	78,400	36,800	1,500	1,100	7,900	6,200	131,900
1933	74,800	52,800	9,400	38,500	20,800	21,600	217,900
1934	55,526	77,520	8,836	16,530	10,330	5,400	174,142
1935	49,963	40,100	10,350	2,100	6,750	21,700	130,963
1936	47,323	34,343	1,850	3,470	9,300	16,200	112,486
1937	200,401	128,331	35,064	11,964	50,515	24,150	450,425
1938	157,956	261,654	17,858	9,393	25,800	33,750	506,411
1939	197,270	245,558	27,115	10,350	78,975	134,715	693,983
1940	72,059	133,300	21,676	16,896	78,176	78,610	400,717
1941	nil	nil	nil	nil	nil	nil	nil
1942	3,500	400	1,000	3,500	4,000	400	12,800
1943	17,400	2,070	4,346	3,720	1,560	1,100	30,196
1944	5,740	46,039	19,913	2,709	7,495	5,890	87,786
1945	5,046	120,832	42,048	13,043	10,750	10,070	201,789
1946	58,509	110,327	69,381	4,655	30,500	5,500	278,872
1947	48,115	151,581	77,403	6,355	4,867	25,000	313,321
1948	88,460	146,835	46,761	7,258	nil	nil	289,314
1949	34,488	59,130	53,129	2,886	9,311	nil	158,944
1950	26,812	50,089	30,916	2,346	1,330	nil	111,493
Totals	3,278,468	3,178,137	641,046	374,275	684,059	684,885	8,840,870
Average per year	117,088	113,505	22,895	13,367	24,430	24,460	315,745
Percentage	37.1	35.9	7.3	4.3	7.7	7.8	100.0

The third or angling period in the fishing of Lac la Ronge was made possible by the construction of a road from the Prince Albert National Park to La Ronge. This road was completed in 1947. In 1948 a small number of anglers visited the lake and many tourist cabins were built. In 1949 about 300 anglers fished the lake. Its reputation spread rapidly, roads and accommodation were improved and in 1950 the first great influx of 3,500 anglers occurred. The need for scientific information on the fish resource had been anticipated. In 1948 the writer began a detailed study of the lake and in 1950 an inclusive creel census was established.

The statistics from 29 years of commercial fishing were of considerable value in planning for the management of the angling fishery. They indicated a proven capacity for annual production of desirable fish of at least 1 lb per acre. The angling catch increased rapidly and there was no doubt that the fishery should be managed primarily for angling. It was clear, however, that the lake could also support an extensive commercial fishery for non-game species. The results of this continuing commercial fishery will be summarized before proceeding to a more detailed consideration of angling for trout.

Commercial fishing results for the 10 years from March, 1950, to March, 1960, are presented in Table XIII. The annual catch averaged about 250,000

TABLE XIII. Commercial fish catch, in pounds dressed weight (head on), from Lac la Ronge in the years 1951 to 1960 (fiscal year ending March 31).

Season	Whitefish	Trout	Pickerel	Pike	Suckers	Others	Total
Summer 1950	nil	nil	nil	nil	nil	nil	nil
Winter 1950–51	55,863	3,155	3,816	3,686	3,950	. . .	70,470
Summer 1951	67,264	86	657	924	42,490	500	111,921
Winter 1951–52	119,622	1,721	1,123	1,090	2,874	725	127,155
Summer 1952	nil	nil	nil	nil	nil	nil	nil
Winter 1952–53	149,034	6,860	993	374	3,285	. . .	160,546
Summer 1953	nil	nil	nil	nil	nil	nil	nil
Winter 1953–54	84,402	6,382	559	391	1,817	800	94,351
Summer 1954	nil	nil	nil	nil	nil	nil	nil
Winter 1954–55	204,673	19,109	2,417	2,574	11,123	41	239,937
Summer 1955	185,415	2,480	938	3,037	25,770	1,124	218,764
Winter 1955–56	108,359	7,810	3,443	641	64	111	120,428
Summer 1956	172,957	4,563	3,391	7,313	26,042	767	215,033
Winter 1956–57	29,948	4,983	1,394	392	. . .	24	36,741
Summer 1957	262,705	7,866	13,079	9,251	1,769	. . .	294,670
Winter 1957–58	58,970	5,438	1,847	436	128	. . .	66,819
Summer 1958	302,954	5,949	25,993	9,645	. . .	. . .	344,541
Winter 1958–59	nil	nil	nil	nil	nil	nil	nil
Summer 1959	311,975	3,907	24,077	4,621	. . .	. . .	344,580
Winter 1959–60	41,873	5,967	1,293	405	. . .	. . .	49,538
Average per year	215,601	8,628	8,502	4,478	11,931	409	249,549
Percentage	86.4	3.5	3.4	1.8	4.8	0.16	

lb as compared to 315,000 in the preceding 29 years. Vigorous efforts were made to increase the whitefish production while keeping the game fish catch to a minimum. The success of this program can be seen in the increase of whitefish from 37.1 to 86.4% of the catch, the decrease of trout from 35.9 to 3.5% and of the three game species combined from 47.4 to 8.7%. This result is especially commendable since the whole program was experimental, with administrators, fisheries biologists, conservation officers and commercial fishermen co-operating in attempts to find times, places and methods by which more whitefish and less game fish could be caught. The catches listed in Table XIII are separated as to summer (including autumn) and winter (which means through the ice, in December to March). In four summers no commercial fishing was carried on and none in the winter of 1958–59. Comparing summer and winter catches it will be seen that the summer operations were much more successful in avoiding game fish. This is attributed both to the greater segregation of species in summer and to the greater ease with which nets could be moved in this season. The catch in the 5 years in which both summer and winter netting was carried on is as follows:

In 5 summer catches averaging 230,000 lbs—trout 1.4%, all game species 6.4%
In 5 winter catches averaging 86,000 lbs —trout 9.7%, all game species 13.6%

Even higher proportions of whitefish and less game fish have been taken in some years by fishing during the spawning season of the whitefish, although heavy weather at this time of year made the operation difficult. With the information now available and with improved fishing boats it should be possible to extend the commercial fishery on Lac la Ronge while taking only negligible quantities of trout and other game fish. Recent observations suggest that, while the pike needs maximum protection, the walleye population may well support a modest commercial limit in addition to the present anglers' catch.

Creel Census

The creel census, begun in 1950, has been carried out each year by a full-time investigator. Since La Ronge is reached by a single highway and all the resort operators are located in one townsite, it has been possible to obtain from 65 to 90% coverage in the 11-year period under review. The creel census procedure was described by Rawson and Atton (1953).

Data concerning the catch of trout and its relation to the total anglers' catch from 1950 to 1960 are presented in Table XIV. The number of anglers fishing Lac la Ronge increased from 3,500 in 1950 to 7,000 in 1953, then decreased to 3,200 in 1957. This decrease was caused mainly by increasing numbers of anglers flying north to fish new lakes from camps on some 20 lakes, scattered over the northern third of the province. In 1958 to 1960 the number of anglers fishing Lac la Ronge has remained near the 11-year average. The catch per angler[4]

[4]Catch per angler, per visit which averaged about 4.5 hours fishing and frequently extended over two days.

TABLE XIV. Creel census records of anglers' catch in Lac la Ronge 1950 to 1960 with details of the lake trout taken.

	Number of anglers	Catch, all species	Catch per angler	Number of trout	Av. weight of trout	Total weight of trout	Trout in anglers' catch
	no.	*lb.*	*lb.*	*no.*	*lb.*	*lb.*	%
1950	3,500	204,000	58.3	8,882	7.35	65,280	32.0
1951	5,700	280,000	49.1	8,796	7.64	67,200	24.0
1952	6,250	251,000	40.1	5,642	6.68	47,690	19.0
1953	7,000	278,000	39.7	7,642	6.73	51,430	18.5
1954	4,700	205,000	43.6	5,272	6.61	34,850	17.0
1955	4,800	144,000	30.0	4,418	7.17	31,680	22.0
1956	3,900	150,000	39.0	4,032	7.44	30,000	20.0
1957	3,200	116,000	36.2	2,724	7.07	19,256	16.6
1958	4,150	150,000	36.1	4,933	8.21	40,500	27.0
1959	4,500	179,000	39.7	5,262	8.13	42,781	23.9
1960	3,800	200,000	52.6	6,427	6.97	44,800	22.4
Av.	4,682	196,091	42.2	5,821	7.27	43,224	22.0

began with 58.3 lb in 1950 and 49.1 in 1951. Reduced daily limits (discussed below) in 1951 and 1952 were involved in this initial decrease in the catch per angler. The catch fluctuated from 1953 to 1955 but remained remarkably stable at 36 to 39 lb from 1956 to 1959. In 1960 it rose sharply to 52.6 lb.

The anglers' catch of lake trout has varied with the number of anglers (fishing effort) but the percentage of trout in the anglers' catch has also varied. In the first two years, 1950 and 1951, the percentage of trout was high; then from 1952 to 1957 it varied from 16.6 to 22%. In the last three years, 1958 to 1960, the percentage has remained somewhat above the long-term average. The average weight per trout through the 11-year period was 7.27 lb, varying from 6.61 in 1954 to 8.21 in 1958. The continued large catch of trout, the stability of their average size and the continued catch of very large (trophy) trout mentioned above (Table IV), all seem to indicate that the trout population of Lac la Ronge is thriving and that present angling regulations are satisfactory for its conservation.

THE ANGLING FISHERY FOR TROUT

Lake trout are taken at Lac la Ronge with ordinary casting and spinning equipment from the break-up of ice until the surface waters of the open lake warm to about 12°C (54°F). This usually occurs about June 20 but may vary by as much as two weeks in different years. Surface fishing is again successful in mid- or late September when the surface water has again cooled to about 13°C (55°F) and trout have come into shallow water. During most of the season the trout are down in the cooler waters and until recent years were taken by conventional deep trolling equipment, metal lures on weighted metal, fibre or nylon lines. About 1957 some of the La Ronge fishing guides began to develop a new method

for catching trout locally termed "jigging". This technique was improved in 1958 and proved so efficient that in the summers of 1959 and 1960, at least 70% of the trout taken from deep water, were caught in this way. It is possible that this development is partly responsible for the increased anglers' trout catch in the years 1958–1960.

In the so-called "jigging" procedure the boat is anchored over a known "trout hole" usually at depths of 20 m (65 ft) or more. Bright metal lures are lowered with casting rod and line until they reach the bottom. They are then "snatched" away from the bottom reeling up quickly, often with a jerking movement. After reeling in about 10 m (30 ft) of line, if no strike occurs, the lure is lowered and the procedure repeated. Conventional deep trolling in the same areas will take considerably fewer trout although most of the very large trout are said to be caught by trolling.

Locations at which the trout are caught have been recorded by the creel census worker throughout the years of investigation. Since the catch in specific localities varies from week to week throughout the season and to a lesser extent from year to year, it is not possible to give the angler infallible instruction. However, the major generalization can be made that, after the early surface fishing fails, trout will be caught in all the areas indicated in Fig. 1 as deeper than 20 m (65 ft). In the early and late season surface fishing Nut Point and Hunter Narrows are favoured locations. In midsummer many trout are caught in the central islands area, e.g., west from Freeman Island to Stony Narrows, north to Bear Island and south and east of Meraste Point. The creel census indicates that the proportion of the total trout catch taken from Hunter Bay (including Hunter Narrows) has varied in different years from 33 to 69% and averages 45%.

Seasonal variation in angling effort and success is indicated in Fig. 8. The points plotted are the averages for bi-weekly periods through 11 summers, 1950 to 1960. Trout fishing in the last half of May is usually determined by the disappearance of the ice. Although the bay at La Ronge clears early and provides fine walleye fishing, the trout waters are often not accessible until the last few days of May. The trout catch is heaviest in June with the catch in the second half usually exceeding that in the first. The catch is still great in July but drops to a low in the second half of August, then rises slightly in September. In 1959 and 1960 September catches were 50% higher than the long-term average. The trout catch per angler throughout August and September is about the same as that in July, and greater than that in the first half of June (Fig. 8). Thus the anglers' preference for June fishing is not based on greater success in catching trout in this season.

The trout and other game fish caught by anglers in Lac la Ronge have always been well handled. The commercial processing plant froze much of the anglers' catch in whole or filletted form from 1950 to 1955. In recent years several of the outfitters operating the larger tourist camps and boat businesses have constructed their own filletting and freezing facilities. The angler from

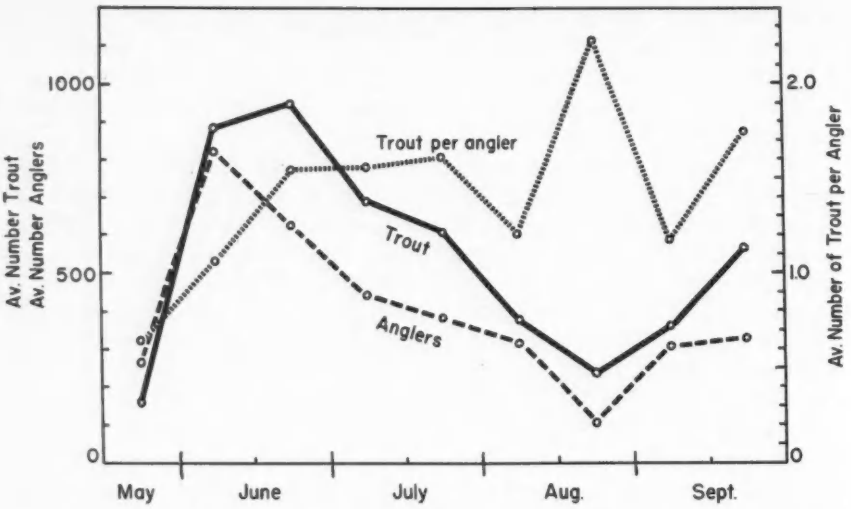

Fig. 8. Seasonal variation in the catch of lake trout, numbers of anglers and trout catch per angler in Lac la Ronge. The values plotted are averages for half-month periods throughout the years 1950 to 1960.

distant areas has thus been able to transport his catch in good condition and has been encouraged in good conservation practice.

The regulations in force when our creel census began in 1950 specified a daily limit per angler of 7 lake trout and a size limit of not less than 15 inches. In 1951 the daily limit was reduced to 5 fish. In 1952 the poundage of lake trout to be taken from Lac la Ronge was also restricted, the limit set being "not more than 4 lake trout not to exceed 25 pounds plus one lake trout". In 1958 the size limit was removed for trout and other species taken by angling. The 1951 and 1952 regulations apparently reduced the catch per angler to a level that could be maintained through the remainder of the 10-year period with minor fluctuations. Thus there was no need to consider further restriction. Such action might well have become necessary, had not the rapid development of "fly-in" fishing in more northern lakes reduced the total number of anglers on Lac la Ronge.

The annual harvest of trout from Lac la Ronge in the years 1950 to 1960 has averaged 43,000 lb taken by anglers (Table XIV) plus 8,000 lb taken in the course of the commercial whitefish operation. This amount, 51,000 lb per year, is only 45% of the average of 113,000 lb sustained by the lake over the period 1922 to 1950.

It may be questioned whether even a very large number of anglers could equal the efficiency of gill nets in catching trout from the lake. There is little doubt, however, that Lac la Ronge would well accommodate twice the number of trout anglers which fished the lake from 1950 to 1960.

ACKNOWLEDGMENTS

The writer is indebted to the Fisheries Branch, Saskatchewan Department of Natural Resources, for the opportunity to carry out this investigation and for complete financial and administrative support. The field data were obtained through vigorous and conscientious efforts of numerous student field assistants. Conservation Officers at La Ronge helped in many ways. Special acknowledgment is due to Mr D. Snell who directed the spawn-taking operations and to Mr F. M. Atton, Provincial Fisheries Biologist, who contributed valuable suggestions for the field program and in the analysis of data.

SUMMARY

1. Lac la Ronge, a lake of 500 square miles (1425 km²) lying across the margin of the Precambrian, provides an unusually favorable environment for the production of the lake trout, *Salvelinus namaycush*.

2. Lake trout spawn on shallow rocky reefs in Lac la Ronge during the first week in October when water temperatures are about 10 to 11°C (50–51.8F). Sexual maturity is usually reached in 6 or 7 years. The bulk of the spawning trout are between 21 and 28 inches fork length. A few mature trout, possibly 10%, fail to spawn in any single year.

3. Growth, as revealed by a sample of 900 trout from Lac la Ronge, is slower than that in small lakes of Ontario and New York State, similar to that in the Great Lakes and Great Slave Lake and faster than that in Great Bear Lake. No difference was found in growth of male and female fish and no change in growth rate in two large samples taken ten years apart. There was, however, a distinct difference in growth rates between trout in the main lake and those in the deeper and more oligotrophic Hunter Bay. Trout in the main lake made faster early growth, then slowed and were overtaken at about 12 years by those from Hunter Bay. A comparable difference was demonstrated by Kennedy (1954) between east and west parts of Great Slave Lake. Length-weight studies show that the trout of Lac la Ronge are well nourished, the larger specimens being considerably heavier than those of equal length and age from Great Slave Lake. Lac la Ronge trout grow to a large size; at least 33 fish between 30 and 41 pounds were caught in the years 1949 to 1960.

4. The main foods of the lake trout in Lac la Ronge are ciscoes, whitefish and ninespine sticklebacks. These with 8 other species of fish made up more than 90% of the food of adult trout. The crustaceans *Mysis relicta* and *Pontoporeia affinis* were important invertebrate foods, especially for the younger trout. The latter ate considerable numbers of sculpins and ninespine sticklebacks.

5. The chief parasites of the lake trout were tape-worms, two in the alimentary canal and a third, *Triaenophorus crassus*, encysted in the flesh. Nematodes and parasitic copepods were also collected.

6. Depth distribution and seasonal movements of lake trout were related primarily to temperature. Extensive gill-netting through several summers showed that trout were widespread from the break-up of ice in late May until the upper water warmed to about 14°C (57.2°F), usually near the end of June. The trout were then concentrated in the water deeper than 20 m (65 ft) and at temperatures mostly less than 10°C (50°F) for the remainder of the summer. They came into shallow water again after the autumn turnover and cooling to about 13°C (55.4°F) in mid- or late September.

7. Tagging of 429 trout and recovery of 60 indicated extensive movements. Twenty-one of those recovered were caught from 4 to 22 miles (6-35 km) from the point of tagging. One-twelfth of those recaptured had moved from the main lake to Hunter Bay or vice versa.

8. The catches from 169 gill net sets suggest the relation of trout numbers and weight to those of other species of fish in Lac la Ronge. In the main lake trout make up 11.5% of the weight of the catch, other piscivores 13.5% and plankton and bottom feeders 75%. Trout make up 26% of the catch in Hunter Bay. The average catch of trout in gill nets in 1958 and 1959 was greater than that in 1948 and 1949 even after making allowance for the change from cotton to nylon nets. The average size of trout caught in nets and their age composition remained unchanged after the 10-year period.

9. Commercial fishing in the 29-year period ending in 1950 took an average of 113,500 lb of trout and a catch of all species of 315,700 lb per year. Commercial fishing has continued in the years 1951 to 1960 with an average catch of 250,000 lb per year but the lake trout taken in this operation have been held to an average of 8,600 lb per year or 3.5% of the catch. In this way the trout has been protected for angling without losing the valuable commercial fishery for whitefish.

10. A continuous creel census shows the average trout catch from 1950 to 1960 to be 5,800 fish weighing 43,000 lb. The catch per angler decreased in 1951 and 1952 because of legal restriction but stabilized at about 36 lb. The number of anglers and the catch of trout has risen in the years 1958, 1959 and 1960. The average weight of trout caught has increased slightly and the number of very large "trophy" trout has been maintained.

11. Since the Lac la Ronge lake trout population appears to be thriving in 1960, and since the present annual catch is about 51,000 lb as compared to 113,000 lb per year taken over an earlier 30-year period, it is suggested that the lake could support an angling catch of trout at least double that which is now taken.

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A Limnological Reconnaissance of a Nova Scotian Brown-water Lake[1]

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ABSTRACT

Summer hydrography and biota of a Nova Scotian brown-water lake are described. A bloom of a dinoflagellate was noted. Zooplankton was plentiful. With a range in pH of 4.3–4.8, mollusks were absent. *Chaoborus* dominated in the poor bottom fauna. Standing crop of fish was low at 19 kg per ha. Yellow perch were most numerous, exhibiting a decline in growth rate to age III, then increasing when the fish reached a size to be piscivorous. Fish-cultural implications are briefly discussed.

INTRODUCTION

The principal lake districts of the Canadian Atlantic provinces of New Brunswick and Nova Scotia lie in igneous rock formations. Waters in these areas are characteristically soft. Frequently they are stained in varying degree by humic extractives, derived from marginal bogs or from drainage of bogs, swamps and soils of the forest floor in the watersheds. Boar's Back Lake, Digby County, Nova Scotia, is among those lakes in which high acidity and colour of water are most extreme for the region.

Boar's Back Lake was treated with copper sulphate in 1936 to destroy fish competitors and predators of brook trout (*Salvelinus fontinalis*) (Rodd, 1937). The writer was associated with personnel of the then Fish Culture Branch of the Department of Fisheries, Ottawa, in this operation, and made sporadic limnological observations during the years 1936–38. Data most pertinent to the copper sulphate treatment have been published (Smith, 1938a, 1940). Since that time chemical characteristics of waters in a number of lakes in New Brunswick and Nova Scotia, including Boar's Back, have been reported (Gorham, 1957; Hayes and Anthony, 1958; Smith, 1952). The following account is presented to associate certain qualitative and quantitative aspects of the biota of Boar's Back Lake with the physical and chemical nature of its waters. Some notion is thereby gained of the feasibility of improving the sport fisheries in acid, brown-water lakes of the area by management techniques such as stocking, destruction of undesirable fish species, and others.

LOCATION AND MORPHOMETRY

Boar's Back is a headwater lake tributary to the Carleton Branch of the Tusket River, southwestern Nova Scotia (65°57′W, 44°09′N) (Fig. 1). A survey bench mark at the lake shows an altitude of 223 feet (68 m) above mean sea level.

The lake receives drainage from a forested area of Precambrian quartzites. Its waters are deeply stained, but it is not a bog lake in the sense that it is peripherally surrounded by a true bog. Rather, much of the shore-line is rocky.

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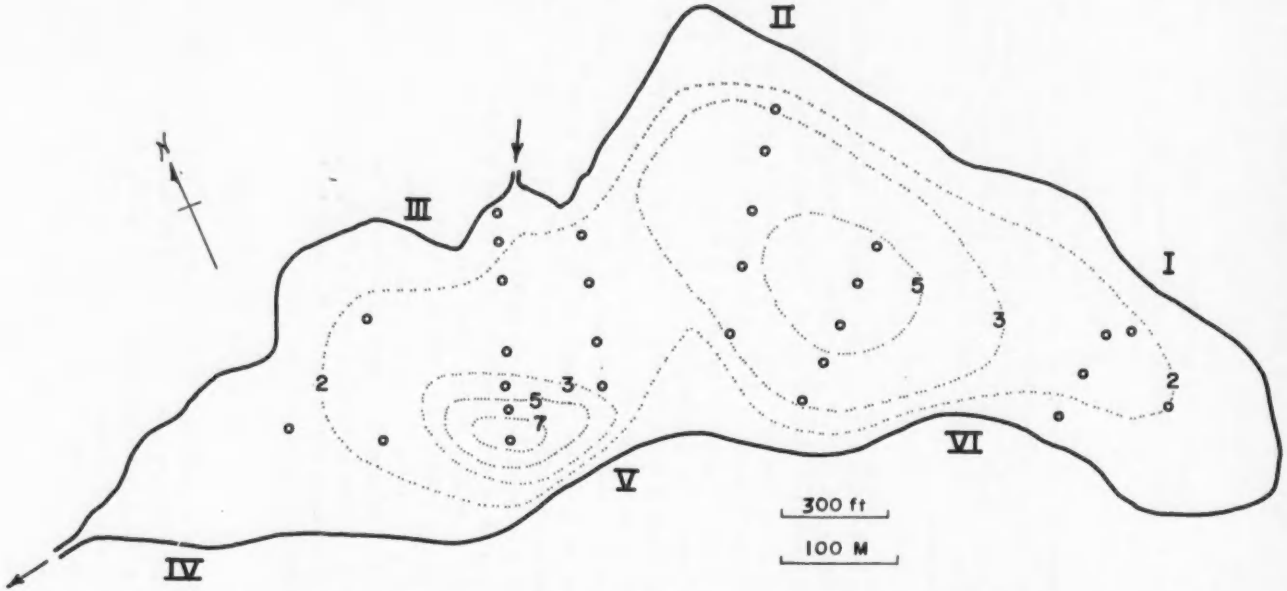

FIG. 1. Map of Boar's Back Lake from a stadia survey by H. A. Lynch, Department of Fisheries, Ottawa, August 1935. Depth contours in metres. Fish collection areas indicated by Roman numerals. Circles indicate bottom fauna stations.

Boar's Back Lake has an area of 55.8 acres (22.6 ha). The maximum depth is 26 feet (8 m), and the mean depth, 8.5 feet (2.6 m). An estimate of volume is 20,655 x 10^3 cubic feet (585 x 10^3 cu m). The shore and volume developments are 1.49 and 1.11 respectively.

HYDROGRAPHY

Chemical characteristics of the surface water of Boar's Back Lake given by Hayes and Anthony (1958) are as follows: pH, 4.7; M.O. alkalinity as ppm $CaCO_3$, 5.7; ppm of Ca, 1.3, of Mg, 1.0, and of Na+K, 4.0; colour as ppm Pt, 163; conductivity in megamhos at 20°C, 41. Conductivity appears high for such acid waters. Hayes and Anthony (1958) found that conductivity did not continue to decline with increasing acidity in acid-water Nova Scotian Lakes, but actually rose when pH values fell below 6. They attribute this seeming anomaly in part to contributions of salt from sea winds. Influence of salt carried by sea breezes and gales in the electrolyte content of coastal fresh waters has been noted widely, not only for Nova Scotia (Gorham, 1957) but elsewhere as well, by Yoshimura (1936) and others.

The writer found that the waters of Boar's Back Lake were thermally stratified in summer (Fig. 2). Bottom water temperature at 8 metres on August

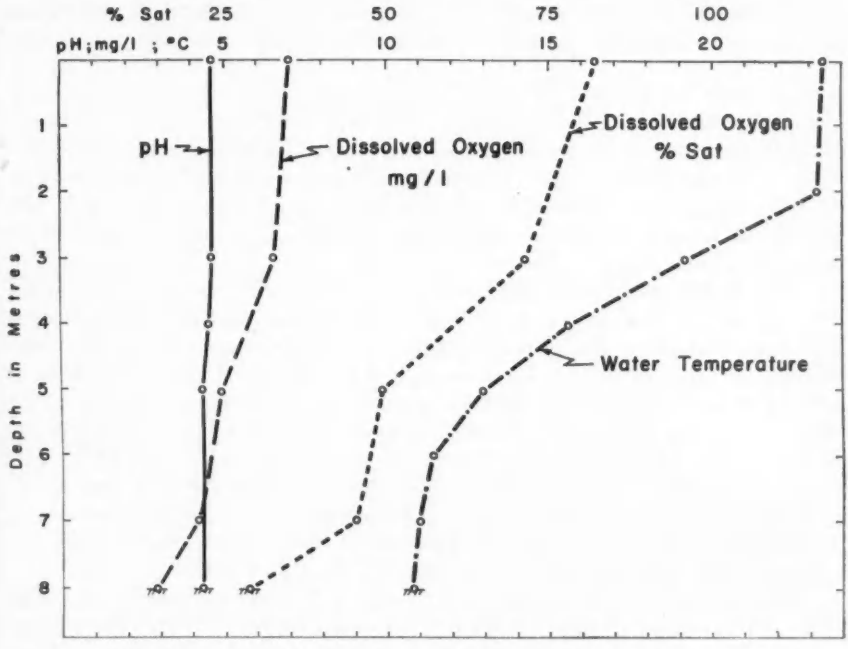

FIG. 2. Temperature, dissolved oxygen and pH values of water in Boar's Back Lake, August 16, 1938.

10, 1936, was 15.4°C. On the same date in 1938 it was 10.8°C. Between these two years at least, there was apparently considerable difference in the temperature level to which the spring period of homothermy persisted, with advance of the open-water season. A higher dissolved oxygen value for bottom water in 1936 than in 1938 (3.8 as against 2.9 mg per litre) also suggests that the homothermous condition persisted to a later date in 1936.

With summer stratification, pH and dissolved oxygen values were reduced in the hypolimnion (Fig. 2). The lowest recorded oxygen content at the bottom was 2.9 mg per litre on August 10, 1938. However, the volume of water with low oxygen values was a small part of the total lake volume. Reduction of dissolved oxygen was not seriously adverse to life in much the greater part of the volume of Boar's Back Lake.

Acid brown-water (dystrophic) lakes may have a good bacterial flora. Hayes and Anthony (1959) found a positive correlation between bacterial counts and colour for Nova Scotian lakes. Diminution of dissolved oxygen in brown-water lakes may be attributed in large part to bacterial decomposition of the allochthonous organic matter which imparts the brown colours. Hutchinson (1957) has pointed out that purely chemical processes, and possibly photo-oxidation, may also be involved. Whatever the processes, there appears general agreement that allochthonous rather than autochthonous organic materials are dominantly concerned. Accordingly, a reduced oxygen content in brown-water lakes, such as Boar's Back, is not considered as an index of eutrophy.

PHYTOPLANKTON

Qualitative phytoplankton samples were collected during July 1936 with a number 20 silk plankton net. Specific identifications of constituents have been reported by Hughes (1949) with the locale designation "N.S. Digby lake". The number of species of algae in the samples was small, as may be seen in the Appendix of this paper.

The dinoflagellate, *Peridinium limbatum*, was dominant in the samples. The species has been reported as rather rare in bogs and soft-water lakes of Wisconsin and Minnesota (Prescott, 1951), and a Charlotte County lake, New Brunswick (Hughes, 1949)., Its occurrence in Boar's Back Lake was noteworthy in that it was sufficiently abundant to form a moderate water-bloom during July 1936. The most visible manifestation of the bloom was a "film" at the surface of the lake when calm in early morning. The phytoplankton of acid brown-water lakes is almost invariably reported to be quantitatively poor, rarely with development of water-blooms (Thienemann, 1925). The blooming of *P. limbatum* under the dystrophic conditions in Boar's Back Lake would thus appear to have been an unusual observation.

Desmid species are prominant qualitatively in the phytoplankton (Caledonian type of algal flora) of lakes of New Brunswick and Nova Scotia that receive drainage from areas of igneous rocks (Smith, 1938b, 1952; Hughes, 1949). However, the number (5) of desmid species found in Boar's Back Lake was decidedly

less than the 51 species recorded for Lake Jesse, and the 25 for Tedford Lake, which are two soft-water lakes also located in southwestern Nova Scotia (Smith, 1938b; Hughes, 1949). The acidity of Boar's Back Lake (pH 4.3–4.7) is definitely higher than encountered in Jesse (pH 6.3–6.6) or in Tedford (pH 5.6–6.4), as is also the colour. The situation in Boar's Back Lake may reflect inadequate sampling temporally. However, Ruttner (1953) noted a decline in number of desmid species in bog habitats of the Lunzer Obersee environs in Austria as the pH values declined from 4–5.5 to 4.0 and below. Accordingly, there are grounds to suspect that the rather extreme dystrophic environment in Boar's Back Lake conditioned an algal flora aberrant, qualitatively and quantitatively, from that which has usually been encountered in less acid, yet soft-water lakes common in the Maritime region.

ZOOPLANKTON

The zooplankton was sampled quantitatively with a Juday 10-litre plankton trap equipped with a number 18 plankton net. Samples were obtained at 4 stations on July 20, 1936. Five hauls with the trap were made at 1-metre depth intervals. The depths of water at the 4 stations, numbered 1 to 4 in Table I, were 1.5, 8, 2 and 4 m. The zooplankters in five 1-millilitre sub-samples, each taken directly from the 50-litre samples, were enumerated and numbers per litre calculated from these counts (Table I). Additional qualitative zooplankton

TABLE I. Zooplankton in Boar's Back Lake, as number of individuals per litre. Asterisks indicate presence but less than one per litre. Blank spaces indicate absence from counts.

Station	Depth	*Daphnia*	*Diaphanosoma*	*Holopedium*	*Bosmina*	*Immature copepods*	*Diaptomus*	*Mesocyclops*	*Polyarthra*	*Kellicottia*	*Keratella*	*Trichocera*	*Chaoborus*
1	0–1	...	37	1	9	12	36	5	37	...	25	8	...
2	0–1	...	38	1	5	42	36	2	6	...	11	1	*
	1–2	...	11	*	4	19	10	1	3	...	3	*	*
	2–3	...	8	*	6	12	5	*	3	*	2	1	*
	3–4	*	9	1	7	3	3	1	1	3	1	...	*
	4–5	1	3	*	2	2	2	3	*	6	1	...	...
	5–6	*	3	...	*	3	1	4	1	10	1	...	1
	6–7	1	3	...	1	7	2	1	1	5	1	...	*
	7–	1	2	...	1	7	1	*	1	4	*	...	*
3	0–1	...	40	2	4	9	29	5	19	...	19	7	*
	1–	...	23	*	5	12	17	4	9	...	14	2	*
4	0–1	...	37	*	11	18	42	2	12	...	9	1	*
	1–2	...	11	1	6	14	16	2	5	...	3	1	...
	2–3	...	7	*	7	7	6	1	3	2	1	1	...
	3–	...	4	2	5	2	3	2	1	3	1	*	...

samples were collected in 1936, and later in 1937 and 1938, with a number 5 plankton net.

A list of zooplankters found in the several samples is given in the Appendix. For the most part the species encountered are common in soft waters of New Brunswick and Nova Scotia (Smith, 1938a, 1952), as well as being more cosmopolitan in distribution. Rotifers were prominent, and this has been generally noted for bog waters (Harnisch, 1929; Gorham, 1931). Cladocerans and copepods, however, appeared to play a more prominent role in the zooplankton of Boar's Back Lake than was observed by Gorham (1931) for both acid and alkaline bog lakes in northern Michigan.

The two diaptomids found in Boar's Back Lake are sharply contrasted in their distribution in the Maritime Provinces. Whereas *Diaptomus minutus* is almost omnipresent in limnetic plankton, *D. pygmaeus* has not been noted elsewhere in the considerable number of Maritime lakes that have received attention. Pennak (1957) has noted that the simultaneous occurrence of two or more species of crustacean zooplankters of the same genus in limnetic communities of small lakes is unusual.

The recorded distribution of *D. pygmaeus* is in ponds and lakes of northeastern United States (Wilson, 1959). Its occurrence in Nova Scotia is thus within the expected geographic range of the species. Although the restricted geographic range and sporadic occurrence of many diaptomids is well known, the seeming isolation of *D. pygmaeus* among Canadian Maritime waters to Boar's Back Lake, with its more extreme dystrophic conditions, suggests a stenokous species, strongly alkaliphobic. Unfortunately, neither Pearse (1906), who first described the species, nor more recent recorders provide chemical data which would permit further evaluation of this view. The seeming isolation of *D. leptopus* var. *piscinae* Forbes in the most acid of the Michigan bog lakes studied by Gorham (1931) is possibly a parallel situation.

Zooplankters were numerous in Boar's Back Lake (Table I). For most species, however, their numbers declined quite sharply with depth. At station 2 the number per litre of surface water approximated 140, while near the bottom at 8 m the number was about 18. *Daphnia* and *Kellicottia* were exceptions in that they were not encountered until a depth of 3–4 m had been reached. Larvae of *Chaoborus* were taken in fair numbers in the plankton samples. *Chaoborus* reacts negatively to light, retreating to deep waters or the bottom in clear-water lakes during the day, then nocturnally migrating toward the surface. Its planktonic occurrence at practically all depths in Boar's Back Lake in daytime was associated with poor light penetration occasioned by the high colour of the water.

The water of Boar's Back Lake to a depth of about one metre contained about 140 macrozooplankters per litre. These represented a dry weight of approximately 300 mg/cu m. We are here concerned with one estimate of standing crop, which is restricted to surface waters. The value of the data for comparisons is definitely limited. Nonetheless, the data indicate, so far as the zooplankton

is involved, at least a mesotrophic level of production (Birge and Juday, 1922). Thienemann (1925) in his systematic characterization of European lakes recorded the zooplankton of dystrophic waters as often being rich.

BOTTOM FAUNA

The bottom fauna was sampled with an Ekman dredge (81 sq in; 522 sq cm) at 30 stations, July 11–17, 1936 (Fig. 1). As much of the bottom materials as possible was washed through a set of three screens of which the finest had a mesh gauge of 1 mm. The organisms, while still alive, were sorted from the remaining debris.

Numbers of bottom organisms taken at various depths are given in Table II.

TABLE II. Number of organisms in the bottom fauna of Boar's Back Lake, July 11–17, 1936. Samples were taken of 522 sq cm area each; figures shown are the total numbers taken in all samples at the depths indicated.

Depth of water (*m*)	0–2	2–3	3–4	4–5	5–6	6–7
Number of samples	8	10	5	3	2	2
Chaoborus larvae	22	122	84	54	33	30
Chaoborus pupae	3	15	24	11	3	3
Chironomid larvae	6	8	5	6	6	11
Caddisfly nymphs	2	10	1	3	1	0
Mayfly nymphs	0	1	0	1	0	0
Damselfly nymphs	0	0	1	0	0	0
Beetle larvae	2	1	0	0	0	0
Asellus sp.	1	0	0	1	0	0
Hyalella azteca	0	2	0	0	0	0
Oligochaeta	3	1	0	1	0	0
Totals	39	160	115	77	43	44
Av. no. per sample	4.4	16.0	23.0	25.7	21.5	22.0
Range	1–28	9–32	15–38	7–43	21–22	21–23
No. per sq m	84	307	441	492	412	422

The summer standing crop of bottom macroorganisms was poor, both qualitatively and quantitatively. To be anticipated from the high acidity and low carbonate content of the water, no mollusks were found in the dredge samples, or by search of the littoral areas. The bottom fauna consisted almost entirely of immature insects. Of these, *Chaoborus* larvae and pupae (84% by number) were dominant. As already noted, *Chaoborus* may be considered both as a bottom organism and as a plankter. In Boar's Back Lake it was found distributed throughout the volume of lake during the day which was the time when the bottom samples were collected. Thus, neither the plankton not bottom samples alone gave a good picture of total numbers of *Chaoborus* in the lake. Based on our samples, and considering that they showed a reasonably representative distribution of *Chaoborus* in Boar's Back Lake on a July day, estimates of numbers in the lake

disclosed that the ratio of these midges as plankton to those as bottom organisms was 2.2:1 (140×10^6 plankton as against 63×10^6 bottom organisms). If *Chaoborus* larvae and pupae were excluded from the bottom fauna, as they would be to a large extent had the bottom samples been taken at night, the summer standing crop of bottom organisms would indeed be small.

Particularly where immature insects dominate, and because of their emergence as adults, summer standing crops of bottom organisms are often minimal for the year. Notwithstanding this probability, our sampling indicates that the bottom fauna of Boar's Back Lake was poor at any season, in agreement with the findings of other investigators of brownwater lakes. The total dry weight of the bottom organisms taken in the 30 samples in July was 0.188 g, or 0.120 g/m².

FISH

Standing Crop

Boar's Back Lake was treated with copper sulphate (3.03 ppm $CuSO_4 . 5H_2O$) on August 5, 1938 (Smith, 1940). Counts of killed fish were made on six shoreline areas, each 85 m (279 ft) in length, selected before the poisoning (Fig. 1). These areas covered about 18% of the lake's perimeter. The fish were enumerated and removed daily for 12 days following the poisoning, that is until very few individuals were found. All fish on certain smaller shore areas, included in the above, were collected and preserved for subsequent length, weight and age determinations.

Seven species of fish were found in the lake, of which yellow perch (*Perca flavescens*) were most plentiful (Appendix). Estimates of the numbers and weight of fish in the standing crop are given in Table III. One may safely assume that the horizontal and vertical distribution in the lake at the time of poisoning differed among the several species. This situation probably affected the relative numbers of the various species found on the sampling areas. Westerly winds prevailed during most of the investigational period. Accordingly, in making population estimates, the perimeter of the lake was arbitrarily divided into three equal sections, eastern, central and western, for each of which two collection areas were considered representative.

It was assumed in making the estimates of standing crop that (1) all fish were killed by the copper sulphate, and (2) they came ashore after being poisoned. There are no data to gauge how well these assumptions were met at Boar's Back Lake. Quite commonly a few fish survive treatment of lakes with copper sulphate or rotenone. Neighbouring Tedford Lake was treated with copper sulphate in 1936. Later in 1953 rotenone was added to this lake under the auspices of the Fish Culture Development Branch of the Canada Department of Fisheries. Killifish, golden shiner, bullhead, and eel were found to have repopulated the lake either from survivors of the first poisoning or from immigrants. However, yellow perch and white perch (*Morone americana*), which had been dominant in the fish population of Tedford Lake in 1936, were absent in 1953. With

TABLE III. Estimates of number and weight of fish in standing crop, Boar's Back Lake, August 1936.

	Brook trout	White sucker	Golden shiner	Brown bullhead	American eel	Banded killifish	Yellow perch
				I—Numbers			
Collection area 1	...	19	52	48	19	80	1,057
2	...	10	27	74	10	35	632
3	...	5	44	54	5	21	349
4	...	11	5	81	11	35	289
5	...	7	17	44	7	16	531
6	...	7	38	79	7	29	1,231
Estimated							
Eastern third of lake	...	143	495	699	143	649	12,584
Central third of lake	...	132	242	649	94	281	6,397
Western third of lake	...	88	270	743	88	308	3,509
Total for lake	23	363	1,007	2,091	325	1,238	22,490]
				II—Weight			
Number in sample for weight	20	19	54	45	12	91	822
Weight of sample	2.84	10.66	0.51	2.29	0.23	0.31	3.63
Calculated for lake kg	3.3	203.7	9.5	106.4	6.2	4.2	99.3
" " " lb	7.3	449.2	20.9	234.6	13.7	9.3	219.0

III—Totals for lake

Number of fish	27,537	Number per ha	1,218
		per acre	493
Weight of fish, kg	432.6	kg per ha	19.1
lb	954.0	lb per acre	17.1

respect to the second assumption, Krumholz (1948) and Ball (1948) were able to recover 84 and 54% respectively of marked fish introduced into small Michigan lakes before poisoning with rotenone. In part, poor recovery was attributed to entanglement of fish in bottom vegetation, and to failure of individuals to float after sinking to the bottom in cool, oxygen-depleted deep water. Such collection conditions were definitely less adverse at Boar's Back Lake, but the estimate of 17 lb per acre (19 kg per ha) for the standing crop of fish should be considered minimal. However, an upward revision, even to doubling the estimated standing crop, would still indicate a small fish biomass (Carlander, 1950).

COMMENTS ON CERTAIN SPECIES

BROOK TROUT. The entire shoreline of Boar's Back Lake was searched for this species on three occasions after the poisoning. Only 23 specimens were found. Nine individuals in a preserved sample of 20 trout appeared to be age II, with a mean fork length and standard deviation of 17.6 $\pm$ 1.5 cm, weight of 66 $\pm$ 11.5 g, and condition index (K) of 1.19 $\pm$ 0.080. For the other 11 specimens in the sample, ranging in length from 21.3 to 30.7 cm and in weight from 126 to 361 g, it was not possible to read their scales with acceptable confidence.

Spawning facilities for trout at Boar's Back Lake, either in tributaries, in the outlet for considerable distance below the lake, or in the lake itself, are very limited, if any exist at all. In view of this, and the absence of young trout, it would appear that the small population consisted of upstream migrants into the

lake. It is not to be inferred, however, that Boar's Back Lake would be appreciably more productive of trout if stock were introduced. Advanced brook trout fry were introduced in the lake after the poisoning, 42,000 in 1938 and 50,000 in 1945 (Rodd, 1940, 1947). In 1956, 4000 fish of the year were planted. Although no creel censuses were subsequently maintained, guides and anglers reported few or no trout taken from Boar's Back Lake after the plantings (Fishery Officers B. H. Comeau, J. H. Thibault, personal communications).

WHITE SUCKER. Numerically suckers held a subordinate position in the fish population. They were relatively large individuals, however, with the result that this species made up a good proportion (47%) of the fish biomass in 1936 (Table III). The fork length range encountered in a sample of 19 fish was 14.3 to 44.0 cm. Ages were not determined, but as might be judged from length frequencies, two year-classes dominated, one (9 specimens) with a mean fork length of 36.4 cm and weight of 578 g, the other (6 specimens) at 41.5 cm and 774 g. The condition index (K) of the larger suckers (33.5 to 44.0 cm) was low, at 1.15 ± 0.182. Carlander (1950) gives a range of 1.70 to 2.26 in average values for this species in several American lakes. There was a very low quantity of bottom fauna to support a bottom-feeder such as the sucker.

Sucker populations in Maritime headwater lakes are often maintained from spawning in outlets. The absence of young suckers indicates that this was not the situation at Boar's Back Lake, for certain years just prior to 1936 at least. Quite possibly suckers in Boar's Back Lake were consistently unsuccessful in maintaining a population in the lake, and, as postulated for brook trout, the population present in 1936 was recruited from larger lakes lower in the river system. Recruitment would seem most probable from spawning fish, some of which continued to move upstream instead of dropping back into the lake of their origin, in this case larger Hunter Lake to which the outlet of Boar's Back Lake is tributary. The possibility is considered since, if true, the low estimated standing crop of 19 kg of fish per hectare, initially viewed as having been produced in Boar's Back Lake, would be materially lower, reduced by a possible maximum of 9 kg/ha if no sucker growth occurred in the lake.

AMERICAN EEL. This species is successful in Maritime lakes generally but, aside from brook trout, it was the least numerous of the fish in Boar's Back Lake (Table III). The relatively small number may in part reflect the unproductive character of the lake. However, Smith and Saunders (1955) noted that smaller standing crops of eels were associated with greater distances of lakes from sea. Lake populations of the catadromous eel are largely recruited at the elver stage from the sea. Boar's Back is a headwater lake with numerous lakes in the river system below, which could hold upward-moving young eels. This situation is advanced as an important factor in the low population of eels in Boar's Back Lake.

YELLOW PERCH. This species made up 82% of the estimated 1936 fish population numerically, but contributed only 23% of the total fish biomass (Table

III). Size for age was small, appreciably smaller than in neighbouring soft-water Lake Jesse (Smith, 1939) and much below that in more eutrophic waters (Carlander, 1950).

The growth pattern for yellow perch in Boar's Back Lake differed from that most often noted for fish populations (Ricker, 1958). However, this pattern can emerge for species, such as perch, which feed on their own young, and are capable thereby of self-regulation of population (Nikolsky, 1956). These are species that by this characteristic in feeding can very well maintain populations when other foods for mature fish are scarce. Data for the perch from Boar's Back Lake appear illustrative of the bionomics of this type of fish population.

The growth rate declined from age 0 to age III, then increased quite sharply, to fall off again amongst the oldest fish (Table IV, Fig. 3). This growth pattern

TABLE IV. Age, length in centimetres, and weight in grams, with standard deviations, of perch from Boar's Back Lake. Age 0 perch were not weighed individually.

Number in sample	Age	Fork length	Weight
126	0	3.7±0.36	0.7
438	I	6.1±0.47	2.4±0.62
107	II	7.3±0.41	4.6±0.93
78	III	8.1±0.41	6.2±1.56
50	IV	9.2±0.76	9.8±2.67
7	V	11.9±0.98	22.8±6.86
3	VI	15.8	56.6
3	VII	19.2	102.8

would seem quite consistent, however, with ecological conditions, particularly with respect to food supply, encountered by the perch, and with the feeding habits of the species. Zooplankters, bottom organisms, and fish serve as food of yellow perch (Pearse and Achtenberg, 1921; Parsons, 1950). In most habitats more productive than Boar's Back Lake, bottom organisms are an important sustaining food source for all except possibly the youngest fish. In general, size of preferred food organisms increases with size of fish. In Boar's Back Lake the supply of zooplankters was quite good, but in contrast bottom organisms were scarce. There would appear to have been a deleterious effect on both growth and survival with increasing age, largely as a result of few bottom organisms. However, the relatively small number of perch that became big enough to be piscivorous found a good source of food in small fish, particularly their own young. The fish food supply was small but so was the number of feeders. Growth rate of the older perch could then increase consistent with, but not reflecting the low level of production in the lake, especially that of bottom organisms. The growth pattern of the perch in Boar's Back Lake provides an example where size rather than age determined growth rates, in this instance

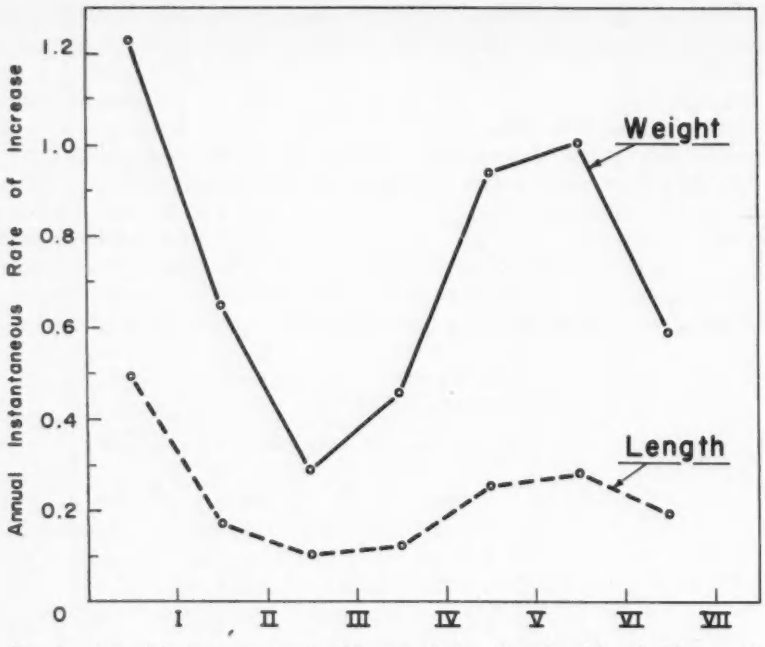

FIG. 3. Annual instantaneous rate of increase in length and weight of yellow perch from Boar's Back Lake.

where there was the "ecological opportunity" when adequate size to feed on fish was attained (Parker and Larkin, 1959).

A growth pattern similar to that for perch in Boar's Back Lake was also evident, but in a much less striking manner, for *Perca flavescens* in Wisconsin lakes (Schneberger, 1935) and for some year classes of *P. fluviatilis* in Windermere (Le Cren, 1958). Bottom organisms can be an important and adequately sustaining source of food for perch populations (Pearse and Achtenberg, 1921; Parsons, 1950). Thus where the bottom fauna is rich, there may be no depression in growth rate of intermediate sizes of perch such as was found in Boar's Back Lake.

DISCUSSION AND SUMMARY

Dystrophy is manifest in varying degrees in a majority of lakes in the eastern Maritime provinces of Canada. Boar's Back is among those lakes of the region exhibiting dystrophic conditions most extremely. Associated with this was a very poor production of bottom fauna and of fish, as might be gauged from the standing crops. However, production appeared better at other trophic levels. A bloom of a dinoflagellate was noted, and zooplankters were numerous, especially

in the epilimnial waters. Although the bloom of the dinoflagellate, *Peridinium limbatum*, at least superficially suggested eutrophy, Margalef (1958) has pointed out these algae actually "immobilize organic matter and slow down the turnover" since they are poorly grazed.

The stocking of dystrophic Boar's Back Lake with the indigenous brook trout did little to improve angling, even when other species were eliminated. Johnson and Hasler (1954) obtained more favourable results from stocking non-indigenous rainbow trout in small dystrophic lakes of Wisconsin. This was done in the knowledge that rainbow trout may feed at all ages on zooplankters which usually predominate in the fauna of dystrophic waters. Rainbow reputedly tolerate higher water temperature than will brook trout. The non-indigenous smallmouth black bass grows well and appears to utilize the productive capacity of dystrophic lakes in southwestern New Brunswick to better advantage than the native brook trout (Smith, 1942).

Stocking non-indigenous species may result in better utilization of the productive capacities of dystrophic lakes by desired sport fish, yet it does not raise the basically low level of production in these waters. Attempts have been made in this latter direction by liming in Wisconsin and Michigan dystrophic lakes (Stross and Hasler, 1960; Waters and Ball, 1957) and in Scottish soft-water lakes (Holden 1959). Favourable but inconsistent results were observed in production at the lower trophic levels, but improved fish production was minor, if at all. On the whole, dystrophic waters still remain a challenge in management for feasible, worthwhile improvement in production.

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APPENDIX

List of organisms identified from Boar's Back Lake.

ALGAE (identified by E. O. Hughes)

Anabaena sp.
Peridinium limbatum (Stokes) Lemm.
Characiopsis cylindricum (Lambert) Lemm.
Dinobryon divergens Imhof.
Asterionella sp.
Tabellaria sp.
Characium stipitatum (Bachmann) Wille.
Mougeotia sp.
Hyalotheca dissiliens (Smith) Vréb.
Micrasterias apiculata (Ehrenb.) Menegh.
M. truncata (Corda) Bréb.
Staurastrum pentacerum (Wolle) G. W. Smith.
Xanthidium antilopaeum var. *hebridarum* W. and G. S. West.

ROTIFERA (identified by G. Morley Neal)

Kellicottia longispina (Kellicott).
Polyarthra trigla Ehr.
Keratella cochlearis (Gosse).
Trichocera sp.

CLADOCERA

Diaphanosoma brachyurum (Liéven).
Holopedium gibberum Zaddach.
Daphnia sp.
Bosmina longispina Leydig.
Polyphemus pediculus (L.).
Acroperus harpae Baird.
Chydonus sphaericus (O.F.M.).
Acantholeberis curvirostris (O.F.M.).
Drepanothrix dentata (Eurén).
Ophryoxus gracilis Sars.

COPEPODA

Diaptomus minutus Lillj.
D. pygmaeus Pearse.
Mesocyclops edax (Forbes).
Macrocyclops albidus (Jurine).

COLEOPTERA (identified by W. J. Brown)

Hydroporus undulatus Say.
Gyrinus lugens Lec.
G. fraternus Coup.
G. dichrous Lec.
Stenelmis crenata Say.
Acryonyx variegatus Gem.

FISHES

Salvelinus fontinalis (Mitchill)—Eastern brook trout
Catostomus commersonni (Lacépède)—White sucker
Notemigonus crysolucas (Mitchill)—Golden shiner
Ameiurus nebulosus (LeSueur)—Brown bullhead
Anguilla rostrata (LeSueur)—American eel
Fundulus diaphanus (LeSueur)—Banded killifish
Perca flavescens (Mitchill)—Yellow perch